Sometimes we don't solve anything, we just re-arrange the mystery.

But that doesn't mean we should stop trying.

As said by Joe Leaphorn in the movie version of "Coyote Waits", by Tony Hillerman

University of Alberta

Response of aspen (*Populus tremuloides* Michx.) seedlings to cold storage, root aeration, and watering regime

by

Lee Anthony Martens

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of *Master of Science*

in

Forest Biology and Management

Department of Renewable Resources

Edmonton, Alberta

Spring 2006

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ABSTRACT

Fall-lifted and cold-stored aspen (*Populus tremuloides* Michx.) seedlings used in reforestation programs frequently show poor growth after spring planting. Morphology, physiology, and growth of nursery-grown container and bareroot stock and 'naturally' regenerating seedlings were investigated prior to, during, and after cold storage. The growth response of aspen seedlings to root aeration and frequency of watering was also studied. The results from these experiments indicate that growth is improved when aspen seedlings were (1) grown in media with a large fraction of fine particles and an infrequent watering regime, (2) kept in cold storage for 75 d, and (3) characterized by high root:shoot ratios (RSR) and high carbohydrate reserves at time of planting. Aspen seedlings grown under more natural conditions had higher levels of root carbohydrate reserves and higher RSR at the end of the first growing season and had greater growth in their second year, compared to nursery-grown stock.

DEDICATION

For my parents, Bill and Shirley Martens.

Thank you for teaching me to appreciate plants, both big and small.

ACKNOWLEDGEMENTS

I thank my co-supervisors, Drs. Victor Lieffers and Simon Landhäusser, for their assistance and support during the course of my studies at the University of Alberta. It is comforting as a graduate student to know that a simple knock on your door was always answered with "come in" and I have benefited immensely from our many ensuing discussions. Thanks are extended to Dr. John Hoddinott, for his helpful insights during the development of experiments and for his review and many suggestions for improving the thesis.

This project would have been difficult to complete without the support and cooperation of many individuals and organizations. I thank the Western Boreal Aspen Corporation (WBAC) for their financial support and their keen interest in all things aspen. Thanks are also extended to NSERC for financial support. In-kind contributions from Tim Gylander (Weyerhaeuser Company Limited), Florance Niemi (Daishowa-Marubeni International Limited), Don Willis (Jiffy Products Limited), Jeff Hoyem (Woodmere Forest Nursery), Persa Zagorac (Beaver Plastics), Larry Lafleur, Brad Lafleur, Barry Wood, and Henny Darago (Smokey Lake Forest Nursery and Lafleur's Cold Storage) were very much appreciated.

Without the enthusiastic assistance of Anna Brown, Erin Smith, Carmen Gustafsson, and Jane Wolken, I would still be washing roots, measuring dry weights, or grinding samples – thank you! Thanks are also extended to Pak Chow for always being so helpful and putting up with the large quantities of samples for carbohydrate analysis, to Dick Puurveen, Ellerslie Research Station, for accommodating last minute requests for equipment, and to the EFM lab group, particularly Heather Fish, Erin Fraser, and Tara Mulak, for making my M.Sc. experience very interesting and most enjoyable. To office mates Kirsten Hannam and Roxanne McMillan, fellow graduate students Mónica Calvo and Josée Thibodeau, and friends Cynthia Strawson and Rob and Sue Gagnons, my heart-felt thanks for the chats, for the dinners, and especially for the laughter. To my hiking companions, Karin Sibilo, Andy Haden, David Chesterman, and Mark Seemann, we have all come a long way since out geography days and it truly has been a pleasure roaming the backcountry with you before and during my Master's experience and I look forward to many years ahead viewing mountain vistas and big hunks of fudge.

Finally, without the support and encouragement of my parents I cannot imagine what I would be doing. Thank you for helping me layout the field experiments at Ellerslie, for potting up plants in the growth chamber on New Years Day 2003, and for always being just a phone call away. Enjoy the rest of your retirement!

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CHAPTER ONE

GENERAL INTRODUCTION

1.1. Distribution and biology of aspen

Aspen (*Populus tremuloides* Michx.) is a shade-intolerant deciduous tree species native to North America. Its natural range extends across Canada and from Alaska in the north to Mexico in the south (Fowells, 1965; Little, 1971). Aspen is most abundant in the boreal region (Perala, 1990) and achieves its best growth rates on moist but well-drained soils (Haeussler & Coates, 1986; Corns, 1989).

The primary means of aspen regeneration is by vegetative (asexual) reproduction as root suckers from adventitious buds on horizontal lateral roots (Maini & Horton, 1966; Steneker, 1976; Kemperman, 1978). Many root suckers or ramets are formed during the first two growing seasons after disturbance. These ramets are genetically identical to the parent root system. Aspen is also capable to sexual reproduction. The trees are dioecious and the flowers are borne on catkins or amets and typically unisexual (Maini, 1968; McDonough, 1979, 1985). Heavy seed crops are produced at 3- to 5-year intervals (Maini, 1968; Perala & Russell, 1983; Farrar, 1995), with a single mature aspen tree capable of producing over a million seeds (Barnes, 1966; Maini, 1968; Schopmeyer, 1974; McDonough, 1985). Seed dispersal generally occurs in late spring; however, seedling establishment success is often low because seeds are viable for only a short period of time (Maini, 1968; Latva-Karjanmaa, Suvanto, Leinonen, & Rita, 2003) and microsite conditions for germination and seedling growth must be optimal (Zasada & Viereck, 1975; McDonough, 1979; Fechner & Burr, 1981; Jones, Kaufmann, & Richardson, 1985).

1.2. Why is there interest in the plantation culture of aspen?

Several factors have combined to prompt interest in the plantation culture of aspen. Aspen has become an important commercial tree species in North America. It is pulped to make high-density paper and newsprint, and stranded for oriented strand board (OSB) production. A number of industrial forest companies operating on the deciduous land base in the Green Area (i.e. public forested lands that are not available for agricultural development other than grazing) of Alberta have identified a pending aspen wood fibre shortage. Because the Green Area is managed for multiple use and Forest Management Agreements (FMA) are area-based (i.e. defined land area) rather than volume-based (i.e. defined annual volume of timber), oil and gas exploration and agricultural (e.g. cattle grazing) and recreational activities have resulted in a loss

of some land for timber production (i.e. a landbase deletion), which can affect a forest company's annual allowable cut (AAC). This trend is expected to continue into the future. In addition, forest companies currently rely on aspen purchased from private landowners operating in the White Area (i.e. land suitable for cultivation) to meet up to 30% of their annual wood fibre needs; however, this source of wood fibre is forecasted to decline in the near future. To mitigate these timber supply concerns, a strategy to establish fast growing short rotation deciduous crops was developed (Lester, 1995). Aspen is capable of high growth rates. Little is known about the growth of aspen of seedling origin (Peterson & Peterson, 1992; Latva-Karjanmaa et al., 2003); however, ramets often grow 1 to 2 m during the first growing season following disturbance. On good sites, aspen of sucker origin can reach 24 m in height and accumulate gross merchantable volumes of 300 m^3 ha^{-1} by age 60 years (Kirby, Bailey, & Gilmour, 1957 in Peterson & Peterson, 1992; Plonski, 1974). Finally, aspen is a desirable tree species for tree improvement. It has a strong genetic component to growth, wood quality, and insect and disease resistance (Navratil, Bella, & Peterson, 1990). Selection of superior trees and breeding can result in gains of 25% in volume growth, 4% in specific gravity of wood, 6% in fibre length, and 45% in *Hypoxylon* canker resistance (Einsphar & Benson, 1967; Einsphar & Winton, 1976). Therefore, aspen grown in plantations has the potential to produce high quality fibre and high quantities of fibre when intensively cultured.

1.3. Aspen planting stock for plantation establishment

A number of techniques have been developed to produce aspen planting stock including direct seeding, root cuttings, root sprout cuttings, greenwood cuttings, and tissue culture (Peterson & Peterson, 1992; Lester, 1995). From an operational forestry perspective, aspen seedling production based on direct seeding is probably the most efficient and least costly means to produce large numbers of plantable seedlings. Direct seeding can produce two general types of planting stock. Container planting stock is seeded in containers filled with peat based growth media amended with perlite and/or vermiculite and summer grown in greenhouses under stress free conditions until they achieve the required height and stem diameter (Burr, 1985; Johnson, Paterson, Leeder, Mansfield, Pinto, & Watson, 1996). Bareroot planting stock is seeded directly in nursery beds (i.e. 1+0 stock) or it is seeded in containers, summer grown in a greenhouse, over winter cold stored, then transplanted into nursery beds (i.e. plug+1 stock) and grown for an additional year (Benson & Dubey, 1972; Fisher & Fancher, 1984; Williams & Hanks, 1994).

Lifting of aspen seedlings occurs in late autumn. Lifting is a nursery operation in which dormant[1] seedlings are loosened and removed from containers or the soil before storage (Sutton & Tinus, 1983). Lifting of container stock involves the removal of intact shoots and root systems and associated growth medium from containers. Lifting of bareroot stock occurs prior to ground freeze and involves a horizontal undercut in which roots are severed by passing a sharp, thin, flat, reciprocating blade horizontally within the nursery bed. Bareroot seedlings are also root pruned. Root pruning is a nursery practice whereby excessively long roots are trimmed at the packing table after lifting from the nursery bed to facilitate packaging for over winter storage and planting (Armson & Sadreika, 1979). After lifting, container and bareroot stock are placed in plastic bags, boxed, and stored frozen (-3°C) over winter. Over winter storage of stock is used to hold seedlings in a dormant state until conditions are suitable for planting at a field site.

1.4. Growth and survival of aspen stock after outplanting

A common observation of container and bareroot aspen seedlings is that a period of reduced survival and slow growth occurs after outplanting (i.e. planting check or transplant shock). Average height growth of nursery-grown aspen stock in plantations during the establishment phase (i.e. first two to three years after outplanting) has ranged from 0.2 m to 0.9 m after the first growing season and 0.24 m to 1.25 m after the third growing season (Table 1.1). First-year height growth is particularly low, especially for seedlings planted in Alberta. Seedling survival during the establishment phase has ranged from 50 % to 98%. Interestingly, the application of post planting silvicultural treatments such as herbicides to control competing vegetation, irrigation, and N-P-K fertilization does not appear to improve the survival and growth of aspen planting stock in plantations (Table 1.1). This suggests that the reduced survival and poor growth of aspen seedlings after outplanting may not be entirely related to post planting site conditions but rather to their morphological characteristics and physiological condition (i.e. quality) at time of planting.

1.5. Seedling quality

A precise definition of seedling quality has proved to be difficult (Puttonen, 1997). Sutton (1980) used seedling performance in relation to forest management objectives to define seedling quality as 'fitness for purpose'. Stape, Gonçalves, and Gonçalves (2001) suggest that quality planting stock has: (1) no sign or symptoms of disease, (2) a sturdy stem and a fibrous

---

[1] Dormancy is defined as "a temporary suspension of visible growth off any plant structure containing a meristem" (Lang, Early, Martin, & Darnell, 1987).

root system free of deformities with an optimal balance between root and shoot mass, (3) sufficient conditioning to withstand cold storage temperatures and a short period without water after planting, and (5) good carbohydrate reserves and mineral nutrient content. Both definitions of seedling quality suggest that the nursery culture of seedlings with good growth potential is necessary for the successful establishment of seedlings after outplanting and may be species and site specific.

Assessments of quality include both seedling material and performance attributes. Material attributes (e.g. stem height and diameter, root to shoot ratio, number of buds, stomatal conductance, stem water potential, carbohydrate reserves, and days to bud break) measure the stress tolerance, avoidance, or resistance potential of seedlings (Ritchie, 1984; Grossnickle, 2000). Performance attributes (e.g. root growth potential and photosynthesis) measure the functional integrity and growth potential of seedlings (Ritchie, 1984; Grossnickle, 2000). Root growth potential (RGP) has become the most common method of assessing seedling quality (Dunsworth, 1997).

1.5.1. Root growth potential

A key to the successful establishment of nursery-grown seedlings is the ability to grow new roots soon after outplanting for water and nutrient uptake (Stone & Shubert, 1959; Ritchie & Dunlap, 1980; Ritchie, 1984; Burdett, 1990). RGP is defined as a seedling's ability to initiate new roots or elongate existing roots under favourable conditions (Simpson & Ritchie, 1997). The test involves growing seedlings under optimal conditions for a set period of time, usually ranging form 28 to 56 d (Farmer, 1975; Webb, 1977; von Althen & Webb, 1978; Farmer, 1979; O'Reilly, Harper, & Keane, 2002; Mortazavi, O'Reilly, & Keane, 2004). If test results are high, then the assumption is that the seedling will have high survival and growth potential (Ritchie, 1985) because it is capable of photosynthesis, the phloem and xylem are functioning, and the biochemical processes needed for root growth are working (Ritchie & Tanaka, 1990; Noland, Mohammed, & Scott, 1997). Seedlings that are unable to grow adequate roots in the RGP test are assumed to have low survival and growth potential because they may be prone to water stress after outplanting (Ritchie, 1985).

A number of factors can influence the development and expression of RGP. Species (O'Reilly et al., 2002), seed lot (Sutton, 1983), and stock type (i.e. root system size) (Ruehle & Kormanik, 1986; Kormanik, Sung, Kormanik, & Zarnoch, 1995; Ponder, 2000) can influence the development of RGP. In addition, RGP is determined by the physiological condition of the seedling at the time of lifting (Farmer, 1975). Nursery managers determine when seedlings are

lifted and the length of time stock is held in cold storage (Marshall, 1985). Length of cold storage interacts with lifting date to determine the RGP of seedlings at time of planting (Ritchie & Dunlap, 1980; Ritchie, 1985; Dunsworth, 1997). After outplanting, the expression of RGP is influenced by the physical and environmental conditions at the field site (Nambiar, 1980; Grossnickle, 2000). Aspen seedlings are held in cold storage for up to seven months, therefore it is important to establish how over winter cold storage duration affects RGP. Over winter storage can also affect carbohydrate reserves, which are an indication of overall seedling health and growth potential (Marshall, 1985).

1.5.2. Carbohydrate reserves

Carbohydrate reserves are necessary for winter survival and initiation of new growth in the spring (Krueger, 1967; Loescher, McCamant, & Keller, 1990). However, critical carbohydrate reserve levels both at the time of lifting and time of outplanting are not known for most commercially important tree species. In Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings, Ritchie (1982) estimated that total non-structural carbohydrate (TNC) levels of at least 10 to 12 % dry mass were needed to ensure seedling survival and adequate growth after outplanting. A decline in carbohydrate levels during cold storage has been reported for many conifer species (Ronco, 1973; McCracken, 1979; Ritchie, 1982; Cannell, Tabbush, Deans, Hollingsworth, Shapard, Philipson, & Murray, 1990; Jiang, Zwiazek, & Macdonald, 1994); however, little is known of cold storage effects on carbohydrate levels for deciduous tree species.

Carbohydrate reserves also seem to play a critical role by increasing the stress resistance of seedlings after outplanting (Ritchie, 1982; Puttonen, 1986). Although spring root growth in sugar maple (*Acer saccharum* Marsh.) may be partly dependent on root starch concentration (Wargo, 1979), European aspen (*P. tremla* L.) seedlings appear to be dependent on current photosynthates produced by the shoot for new root growth (Eliasson, 1968). Therefore, the time between bud flush and initial root growth is critical for newly outplanted seedlings (Johnson, Novinger, & Mares, 1984). The time between bud flush and initial root growth can be delayed by cold soil temperatures. Cold soil temperatures of less than 5 to 10°C (Larson, 1970; Landhäusser, Desrochers, & Lieffers, 2001) are known to inhibit seedling root growth. In addition, by the time soil temperatures are adequate for root growth soil moisture conditions may cause plant moisture stress, which can limit photosynthesis and root growth (Larson & Whitmore, 1970; Struve, 1990). Adequate carbohydrate reserve levels may buffer seedlings against these stressful conditions (Marshall, 1985) because they are better able to adjust osmotically and maintain cell turgor at low xylem water potentials (Ritchie, 1982).

1.5.3. Dormancy and freezing tolerance

In assessing seedlings for quality, it is important to know their stage in the dormancy cycle (Day & Butson, 1989). Dormancy has two important stages: ecodormancy and endodormancy (Lang, Early, Martin, & Darnell, 1987). Ecodormancy is the earliest stage of dormancy. Seedling growth during this stage is limited by photoperiod and temperature. This acquisition of dormancy brought on by a decrease in photoperiod is necessary for the initial development of freezing tolerance. Freezing tolerance is defined as "the lowest temperature below the freezing point that a tissue can be exposed to without damage" (Grossnickle, 2000). During this stage, seedlings begin to initiate bud set and are able to withstand temperatures >0°C (Levitt, 1980). RGP is moderately high during this period (Day & Butson, 1989) and carbohydrates begin to accumulate (Landhäusser & Lieffers, 2003). The next stage in the development of freezing tolerance begins when seedlings are exposure to temperatures <0°C (Calmé, Bigras, Margolis, & Herbert, 1994), which intensifies dormancy and the number of days to bud break increases. This period of dormancy is called endodormancy and is controlled by the physiological condition of the buds. Seedlings reach their maximum state of dormancy when the number of days required for bud flush peaks (Grossnickle, Parker, Blake, & Sutton, 2001). Endodormancy and maximum freezing tolerance ends when buds have accumulated the required amount of chilling (Lavender, 1985; Burr, 1990). The chilling requirement is the temperature and time required to end endodormancy (Grossnickle, 2000). The chilling requirements of aspen are not known. The chilling requirement of spruce, an important boreal tree species, was reached after between 750 and 1300 hrs at temperatures <5°C (Ritchie, Roden, & Kleyn, 1985; Silim & Lavender, 1991). When the chilling requirements of buds have been met, there is a return to ecodormancy. RGP is highest during the transition from endodormancy to ecodormancy. Ecodormancy ends when environmental conditions are favourable to initiate growth.

1.6. Research questions, objectives, and hypotheses

The focus of this thesis research was to investigate possible reasons for the reduced survival and poor growth of aspen seedlings in plantations. An understanding of the morphology and physiology (i.e. quality) of both artificially produced and 'naturally' (i.e. unrestricted root systems) regenerated seedlings may be key to improving regeneration success. Therefore, **What is the morphology, physiology, and growth of nursery-grown container and bareroot stock and 'naturally' regenerating seedlings prior to, during, and after over winter storage?** The objectives and hypotheses of the experiments are:

1. To determine the morphological and physiological characteristics (i.e. quality) of nursery-grown container and bareroot stock and 'naturally' regenerating aspen seedlings.
   Hypothesis: There is no difference between the morphology and physiology of nursery-grown container and bareroot stock and 'naturally' regenerating aspen seedlings.
2. To determine the effects of long-term over winter cold storage on aspen seedling quality (i.e. root growth potential and carbohydrate reserves)
   Hypothesis: Aspen seedling quality will not change with increasing duration of over winter storage, regardless of stock size.
3. To determine the shoot and root growth of nursery-grown container and bareroot aspen stock and 'naturally' regenerating aspen seedlings one growing season after outplanting.
   Hypothesis: Shoot and root growth of nursery-grown stock and 'naturally' regenerating seedlings will not differ one growing season after planting.

An important result from the above study was that aspen seedlings with high root to shoot ratios (RSR) performed better than seedling with low RSR. Root to shoot ratio is defined as the dry mass of the root system relative to the dry mass of the shoot. Seedlings with high RSR are said to have a high potential for avoidance of water stress because there are plenty of roots for water uptake to off set the transpiration losses from leaves (Bernier, Lamhamedi, & Simpson, 1995; Edwards, 1998). Nursery practices can affect root system development (Davis & Jacobs, 2005), particularly the physical properties of the growth media and the irrigation strategy (Heiskanen, 1993). Therefore,

How important is growing media aeration and watering regime for producing quality root systems in container grown aspen seedlings?

The objectives and hypotheses of the experiment are:

1. To determine the effects of growth media aeration and watering regime on water relations, carbohydrate status and growth of aspen seedlings in containers.
   Hypothesis: Growth media aeration and watering regime will not affect shoot and root growth, leaf area development, root morphology, water relations, and carbohydrate status of aspen seedlings grown in containers.

The following two chapters describe experiments undertaken (1) to determine the over winter cold storage tolerance and first-year growth of nursery grown and 'naturally' regenerating aspen seedlings (Chapter Two) and (2) to determine the growth of aspen seedlings in response to root aeration and watering regime (Chapter Three). These research chapters are presented in paper format. Summary of results, implications for forest management, limitations of the

experiments, and future research needs are presented in the final chapter (Chapter Four). The literature cited format follows the *Scandinavian Journal of Forest Research* (2005).

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Table 1.1. Summary of nursery-grown aspen seedling height growth and survival in plantations.

Stock type	Treatment	Height growth (m)			Survival	Plantation
		Year 1	Year 2	Year 3	(%)	location
Bareroot 1+0	Control	0.43	0.43	0.24	90	Wisconsin[1]
	Simazine	0.34	0.34	0.32	80	
Bareroot		0.30	0.70		50	Michigan[2]
Container (750 ml)		0.49	0.62		87	
Bareroot 1+0	Hand weed	0.90	1.25	1.25	98	Michigan[3]
	Simazine	0.67	1.02	1.02	62	
	Diuron	0.76	1.07	1.07	77	
	Linuron	0.69	1.06	1.06	55	
Various	Various	0.52	0.69	0.95	74	Minnesota[4]
Container (160 ml)	Control	0.27	0.27	0.51	88	Alberta[5]
	Irrigation	0.20	0.20	0.69	80	
	Fertilization	0.26	0.26	0.49	71	
	Irrig + Fert	0.33	0.33	0.67	83	

[1]Benson & Einsphar, 1964
[2]Okafo & Hanover, 1978
[3]Reighard, Howe, & Hanover, 1985
[4]Johnson, 1997; average from 13 plantations established by the Aspen Larch Genetics Cooperative
[5]van den Driessche, Rude, & Martens, 2003

CHAPTER TWO

OVER WINTER STORAGE TOLERANCE AND FIRST-YEAR GROWTH OF NURSERY-GROWN AND 'NATURALLY' REGENERATING ASPEN (*POPULUS TREMULOIDES* MICHX.) SEEDLINGS[2]

2.1. Introduction

Aspen (*Populus tremuloides* Michx.) seedlings used to establish plantations in boreal regions are primarily nursery-grown container and bareroot stock. Seedlings are generally summer grown, fall-lifted, and stored frozen (-3°C) over winter for up to seven months under controlled conditions. A common observation of aspen planting stock is their reduced survival and slow growth after outplanting (e.g. Steneker, 1976; Okafo & Hanover, 1978; Johnson, 1997). This transplant shock (Watson, 1986) may last for up to three years after planting and can occur even in the presence of vegetation control (Benson & Einsphar, 1964; Reighard, Howe, & Hanover, 1985) and irrigation and fertilization (van den Driessche, Rude, & Martens, 2003). This suggests that the reduced survival and poor growth of over winter cold stored aspen seedlings after outplanting may not be entirely related to site conditions but rather to their morphological characteristics and physiological condition at time of planting.

Morphological characteristics determine a seedling's ability to overcome the factors restricting growth at the planting site (Nambiar, 1980; Newton, Cole, & White, 1993; Lamhamedi, Bernier, Hébert, & Jobidon, 1998). Stock size and root system morphology have been shown to be important in determining seedling establishment success after outplanting. There is general consensus that large planting stock remains larger than small planting stock for several years after outplanting (van den Driessche, 1984, 1992; Hashizume & Han, 1993; South & Mason, 1993; Aphalo & Rikala, 2003); however, absolute growth rates may be similar once both large and small stock over come the site conditions restricting growth (Zaczek, Steiner, & Bowersox, 1996). Root system morphology (i.e. number of first order laterals) has been found to be important for seedling survival and growth after planting for sweetgum (*Liquidambar styraciflua* L.) (Kormanik, 1986), northern red oak (*Quercus rubra* L.) (Thompson & Schultz, 1995), white oak (*Q. alba* L.), and black walnut (*Juglans nigra* L.) (Schultz & Thompson, 1990). Appropriate stock size and root system morphology for the successful establishment of aspen seedlings after planting have not been investigated.

[2] A version of this chapter has been submitted for publication in *New Forests*.

The physiological condition of seedlings is known to influence their stress resistance and their ability to successfully establish soon after planting (Ministry of Forests, 1998). Root growth potential (RGP), water relations, and carbohydrate reserves are important physiological attributes of seedlings determining planting stress resistance (Ritchie, 1984). Seedlings must be physiologically ready to grow new roots soon after planting (Ritchie, 1984; Grossnickle & Blake, 1985) for water and nutrient uptake to support photosynthesis (Burdett, 1990). In aspen, the energy for bud flush and early leaf development likely comes from carbohydrate reserves (Landhäusser & Lieffers, 2003), whereas current photosynthates may be necessary for new root growth (Eliasson, 1968). However, for seedlings that are over wintered, RGP, water relations, and carbohydrate reserves may change between the time of lifting and the time of planting (McKay, 1997). The magnitude of the change may depend on the physiological condition of seedlings at time of lifting, the storage environment, and the duration of storage (Camm, Goetze, Silim, & Lavender, 1994). The effect of long-term over winter cold storage on the physiological condition of aspen seedlings is not well understood.

The objectives of the study were to determine (1) the effect of duration of over winter cold storage on aspen seedling RGP, water relations, and carbohydrate reserves, (2) the effect of stock size on the tolerance of aspen seedlings to over winter cold storage, and (3) the importance of seedling morphology and physiology at time of planting for the first-year survival and growth of aspen.

2.2. Materials and methods

2.2.1. Plant material and over winter cold storage

Aspen seedlings used in the study were nursery-grown container and bareroot stock, as well as 'naturally' regenerating (NR; i.e. seedlings with unrestricted shoot and root growth and intact root systems) seedlings. Using two open-pollinated seed sources (Edson, Alberta, 53°27′N, 116°45′W, elevation 1003 m; and, Peace River, Alberta, 57°02′N, 117°42′W, elevation 349 m), containerized aspen seedlings were grown operationally as 1+0 stock at two commercial nurseries (K&C Silviculture, Joffre Alberta, 52°19′N, 113°31′W, elevation 882 m; and Woodmere Forest Nursery, Fairview Alberta, 56°04′N, 118°24′W, elevation 664 m). At each nursery, seeds were sown into PSB 410A (cavity depth 105 mm; diameter 36 mm; volume 80 ml; density 530 seedlings m^{-1}; Beaver Plastics Ltd., Edmonton, Alberta) and PSB 512A (cavity depth 119 mm; diameter 51 mm; volume 220 ml; and density 284 seedlings m^{-1}) styroblock containers filled with peat based growth media amended with vermiculite and perlite. The hard-walls of the containers were ribbed to prevent root spiralling. Seeds were sown on 26 June 2002 at Woodmere Nursery

and 27 July 2002 at K&C Nursery. Container stock was lifted by hand at K&C Nursery on 7 December and at Woodmere Nursery on 10 December 2002. At the time of lifting, container seedlings meeting pre-determined height (PSB 410A, minimum height 13 cm, target height 20 cm, maximum height 45 cm; PSB 512A, minimum height 18 cm, target height 28 cm, maximum height 60 cm) and stem diameter (PSB 410A, minimum diameter 2.4 mm, target diameter 3.2 mm; PSB 512A, minimum diameter 3.0 mm, target diameter 4.0 mm) specifications were randomized within each container type prior to being packed in plastic bags and placed inside wax-coated cardboard boxes.

Bareroot seedlings were grown operationally as plug + 1 (BR P+1) stock at Smoky Lake Forest Nursery (Smoky Lake, Alberta, 54°06′N, 112°28′W, elevation 642 m). Plugs were over winter cold stored PSB 512A container stock grown at Woodmere Nursery during 2001 using the Peace River seed source. Seedlings were transplanted by hand to a sandy loam based bareroot field on 29 May 2002. Spacing was 0.5 m × 0.5 m. Seedlings were grown for one growing season and under cut once to *ca* 0.3 m depth prior to hand lifting on 16 October 2002. Frozen ground would have prevented a later lifting date. At lifting, roots were pruned to *ca* 22 cm below the root collar. Seedlings were packed in plastic bags and boxed. To keep roots from drying out, moist peat was added to the boxes. Due to a delay in opening the cold storage facility, seedlings were placed in cool storage (+3°C) under no light conditions for 15 d.

All container and BR P+1 stock was shipped to the same commercial facility (Lafleur's Cold Storage, Stony Plain, Alberta, 53°31′N, 114°00′W, elevation 704 m) for over winter cold storage. Between 1 November 2002 when BR P+1 stock was placed in over winter cold storage and 9 May 2003 when all container and BR P+1 stock were removed from over winter cold storage, the temperature inside the planting boxes averaged -3.2°C (range -5.8 to -0.2°C).

NR seedlings were grown outside at Ellerslie Research Station, Edmonton, Alberta (53°24′N, 113°53′W, elevation 704 m). To facilitate the eventual extraction of whole seedlings, an area 8.0 m × 8.0 m × 0.3 m was excavated and landscape fabric was laid down prior to filling the excavated area with a 1:1 (v:v) peat and fill sand mixture. To improve germination success, seedlings were pre-established in small soft-walled containers. On 5 June 2002, aspen seeds from the same Edson and Peace River seed sources used to grow the nursery stock were sown into 200 peat based Jiffy 10 mm pellets (pellet depth, 2.0 cm; diameter, 1.0 cm; volume, 1.6 ml; Jiffy Products, Shippagan, New Brunswick). This date was selected as it coincided with the approximate time of germination of wild aspen seedlings. Pellets were placed in a growth chamber providing a temperature of 22°C day/18°C night, 18 h photoperiod, and 60% relative humidity. Light was supplied by fluorescent lamps (Sylvannia F48T12 SHA/VHO, Sylvannia

USA) with 400 µmol $m^{-2}s^{-1}$ photosynthetic active radiation at seedling height. After 14 d, germinants were placed outside under shade cloth (*ca* 40 % light reduction) for 4 d to acclimate to natural light conditions. On 23 June 2002, 120 seedlings of relatively uniform size were transplanted into the excavated area. Spacing was 0.5 m × 0.5 m. Seedlings were not fertilized in 2002 but were watered as needed. Seedlings grew, hardened off and over wintered outside under natural conditions. Between 29 October 2002, prior to ground freeze, and 23 April 2003, after ground thaw, air and soil temperatures were recorded at a weather station located 800 m from the excavated area. Average daily air temperature at 1.4 m above ground surface was -6.7°C (range -30.9 to +14.2°C) and soil temperature at 5 cm depth was -2.8°C (range -10.6 to +7.7°C).

2.2.2. Over-winter cold storage duration treatments

Nursery-grown stock was sampled prior to over winter cold storage (0 d), after 2, 10, and 75 d of over winter cold storage, and prior to field outplanting. Sampling occurred between November 2002 and May 2003 (190 d of over winter cold storage) for BR P+1 stock and between December 2002 and May 2003 (150 d of over winter cold storage) for container stock. At each sampling interval, 88 seedling per container type (PSB 410A and PSB 512A) and 22 BR P+1 seedlings were removed from over winter cold storage and thawed slowly under no light conditions at +3°C for 4 d. Thawing seedlings in this manner represents current practices for conditioning over winter cold stored aspen nursery stock prior to field outplanting. NR seedlings were sampled prior to ground freeze in late October 2002 and immediately prior to bud flush in late April 2003 (*ca* 170 d). Frozen ground prevented a mid-winter sampling. At each sampling interval, 10 seedlings were gently extracted from the soil.

2.2.3. Carbohydrate status

After gently washing roots to remove growth media, 40 PSB 410A seedlings, 40 PSB 512A seedlings, 10 BR P+1 seedlings, and 10 NR seedlings per over winter storage duration treatment were measured for stem height, stem diameter, and root volume (Burdett 1979; Harrington, Mexal, & Fisher, 1994). Root volume was measured by placing a 2 l beaker filled two-thirds full of water on a metric digital scale. After the scale was zeroed, roots were placed in the water and the scale read in grams with 1.0 g equal to 1.0 ml. Stem and root dry mass were also determined after oven drying at 68°C for 48 h. Stem and root tissue sugar and starch concentration were determined using the methods described by Chow and Landhäusser (2004). Dried stem and root tissue were ground separately using a Willey mill to pass a 40-mesh. Sugars were extracted using 80% hot ethanol. Sugar concentrations were then determined

colorimetrically using phenol-sulfuric acid. Absorbance was read at 490 nm. The remaining residue was analyzed for starch by adding sodium hydroxide, enzymatic digestion to glucose hydrolyzate using α-amylase and amyloglucosidase, followed by colorimetric measurement using peroxidase-glucose oxidase/*o*-dianisidine reagent. Absorbance was read at 525 nm.

2.2.4. Growth measurements

At each sampling from over winter cold storage, 48 seedlings from each container type and 12 BR P+1 seedlings were potted and allowed to grow for 30 d (see Webb, 1977) to evaluate their dormancy status and their growth. NR seedlings were not assessed. Seedlings were measured for height and stem diameter prior to transplanting into 1.7 l plastic pots for container stock and 4.5 l plastic pots for BR P+1 stock. All pots were filled with a 3:1 (v:v) mixture of peat (Pro-Mix HP, 65-75% by volume Canadian sphagnum peat moss, perlite, dolomitic and calcitic limestone, and wetting agent) and washed sand. Original nursery growth medium was not washed from roots prior to transplanting. Pots were placed in a growth chamber providing a temperature of 22°C day/16°C night, 18 h photoperiod, light intensity of 400 μmol m^{-2} s^{-1}, and 60% relative humidity. Seedlings were randomized weekly and watered as needed but not fertilized. All pots were thoroughly watered one day prior to final growth measurements.

Each seedling was evaluated daily for initial break of terminal or lateral buds. At the end of each 30 d test, stomatal conductance was measured on the uppermost fully expanded leaf of each seedling at ambient light and temperature between 09:00 and 15:00 hrs using a steady state porometer (LI-1600, Li-Cor Inc., Lincoln, Nebraska). Immediately after the measurement of stomatal conductance, the water potential of the new stem was determined using a Schölander pressure chamber (PMS Instruments, Corvallis, Oregon). Seedling growth over the 30 d period was determined by measuring projected leaf area (LI-3100, Li-Cor Inc., Lincoln, Nebraska), stem and leaf dry mass after oven drying at 68°C for 48 h, and total root volume after washing all growth medium from roots. All roots were stored under no light conditions at +3°C and washed within two days of completion of each 30 d test. Root growth potential (RGP) was determined by subtracting the average volume of roots of seedlings used for carbohydrate analyses from total root volume for individual seedlings after 30 d of growth. Only volume of roots from the same stock type and sampling period combination was used to calculate RGP.

2.2.5. Field performance

The three different types of nursery stock were outplanted *ca* 15.0 m from the area containing the NR seedlings at Ellerslie Research Station. In November 2002, 90 holes 0.3 m in

diameter × 0.3 m deep were drilled into the soil using a 0.3 m auger mounted on a bobcat. Holes were spaced at 1.2 m × 1.2 m, so that there was 1 m spacing to facilitate weed control. Five-gallon blow-molded nursery container pots (Type 1600C, Westgro Horticultural Supplies Inc., Calgary, Alberta) were placed in the holes and filled with a 1:1 (v:v) mixture of peat and fill sand (from same mixture used to grow NR seedlings).

Planting of nursery seedlings in the field experiment coincided with the final sampling from over winter cold storage (see *Over winter cold storage duration treatments* above). After thawing, seedlings were individually planted into plastic pots on 18 May 2003. From time of planting to mid-September, nursery-grown stock and NR seedlings were watered and fertilized weekly using a standard rate of 150 ppm N, 100 ppm P, and 150 ppm K (20-8-20 N-P-K with chelated micronutrients, Plant Products Co. Ltd., Brampton, Ontario and technical grade monopotassium phosphate 0-52-34 N-P-K, Haifa Chemicals Ltd., Haifa, Israel) until bud set. Between bud set and the first hard frost, N was reduced to 50 ppm. Within the plastic pots and excavated area containing the NR seedlings, weeds were removed manually and the area between pots was mowed.

Immediately after planting and at the end of one growing season, all seedlings were measured for height and stem diameter (1 cm above ground surface). After the first hard frost (air temperature < -7°C), all leaves were removed for leaf area and dry mass determination. Leaves were still attached to the stem but were not desiccated. In mid-October 2003 immediately prior to ground freeze, stems were removed, and all growing medium was gently washed from root systems and dry mass and carbohydrate concentration determined. Performance was measured as survival and height, stem diameter, and stem and root dry mass growth.

2.2.6. Experimental designs and statistical analyses

The seedling carbohydrate status, 30 d seedling growth, and field outplanting experiments utilized fully randomized designs with single seedling replicates. The carbohydrate status experiment was analyzed as a 4 × 5 factorial design with 4 seedling types (PSB 410A, PSB 512A, BR P+1, and NR) and 5 collections from over winter cold storage (prior to over winter cold storage, after 2, 10, and 75 d of over winter cold storage, and prior to field outplanting) as fixed effects. The 30 d seedling growth experiment utilized a similar design but did not include NR seedlings (i.e. 3 seedling types × 5 collections from over winter cold storage). The field performance experiment included only the four seedling types.

Differences in carbohydrate concentration, stomatal conductance, stem water potential, and seedling growth data among treatments were tested by analysis of variance (ANOVA) using

the MIXED procedure of SAS (SAS 9.1, SAS Institute, Cary, North Carolina). To meet the assumptions of normality of the distribution of residuals and homogeneity of variance, stomatal conductance, stem water potential, leaf area, and leader, stem diameter, and root growth were log transformed while percentage data were arcsine square root transformed prior to analyses. For presentation purposes, non-transformed means were used. A significance level of $\alpha = 0.05$ was used for all analyses. When significant treatment effects were detected, differences among means were determined by pair-wise comparisons made with the DIFF option. The Tukey adjustment option was used to protect the experiment-wise error rate.

2.3. Results

2.3.1. Pre-storage seedling morphology

Seedling height, stem diameter, total dry mass, and root to shoot ratio (RSR) were affected by seedling type ($P < 0.001$). Height of bareroot P+1 (BR P+1) stock was 51 cm while height of container stock averaged 34 cm and 'naturally' regenerating (NR) seedlings 3.2 cm (Table 2.1). Stem diameter of BR P+1 stock averaged 6.77 mm, which was 3.24 mm larger than container stock (average 3.53 mm) and 5.56 mm larger than NR seedlings (1.21 mm). Total seedling dry mass of BR P+1 stock was 15.5 g while total seedling dry mass of PSB 512A stock was 2.2 g, PSB 410A stock was 1.1 g, and NR seedlings was 0.4 g. NR seedlings had the highest RSR (6.6) while PSB 410A had the lowest RSR (0.8).

2.3.2. Effects of cold storage duration on seedling carbohydrate status

Stem and root sugar, starch, and total non-structural carbohydrate (TNC) concentrations were affected by seedling type ($P < 0.001$), over winter cold storage duration ($P < 0.05$; except for stem starch $P = 0.294$), and their interaction ($P < 0.001$). While in over winter cold storage, there was an overall decline in stem sugar, root starch, and stem and root TNC concentrations in all seedling types (Figures 2.1A, D, E, and F). BR P+1 and PSB 512A stock had the largest decline in stem sugar concentration (3% of dry mass) while NR and PSB 410A seedlings had the smallest decline in stem sugar concentration (2% of dry mass). NR seedlings had the greatest decline in root starch (6% of dry mass) and stem TNC (4% of dry mass) concentration while container stock had the smallest decline in root starch (average 3% of dry mass) and stem TNC (average 2% dry mass). Root sugar concentrations declined in container stock (average 4% of dry mass), while root sugar concentrations did not change for BR P+1 stock and NR seedlings (Figure 2.1B).

Stem sugar concentrations of PSB 512A stock decreased during the first 10 d of over winter cold storage while PSB 410A and BR P+1 stock were unaffected. Thereafter, stem sugar concentration remained relatively constant in container stock while stem sugar concentrations declined further in BR P+1 stock from 12% of dry mass after 75 d of over winter cold storage to 9% of dry mass prior to spring outplanting. Root sugar concentrations increased during the first 10 d of over winter cold storage for PSB 410A, PSB 512A, and BR P+1 stock then declined thereafter (Figure 2.1B). Root starch concentration showed an opposite trend. During the first 10 d of over winter cold storage, root starch concentration decreased in all nursery-grown stock then remained relatively constant thereafter for container stock. Root starch concentration increased in BR P+1 stock from 4% dry mass after 75 d of over winter cold storage to 9% of dry mass prior to spring outplanting. Prior to the commencement of growth in the spring of 2003, NR seedlings had the highest stem and root sugar, starch, and TNC concentrations. PSB 410A stock had the lowest root sugar and root TNC concentrations while BR P+1 stock have the lowest stem sugar, stem starch, and stem TNC concentrations.

2.3.3. Growth in relation to cold storage duration

Endodormancy (Lang, Early, Martin, & Darnell, 1987) occurred prior to over winter cold storage for all nursery-grown stock. Prior to over winter cold storage, BR P+1 stock required >21 d for initial bud flush while PSB 512A stock averaged 9 d and PSB 410A averaged 8 d to initial bud flush (Figure 2.2). Prior to spring outplanting, all nursery-grown container and BR P+1 stock averaged 5 d to initial bud flush.

Differences in stem dieback and total leaf area were due to stock type ($P < 0.001$), over winter cold storage duration ($P < 0.001$), and their interaction ($P < 0.001$). For all nursery-grown stock, stem dieback was lowest prior to over winter cold storage (0%) and highest prior to field outplanting, especially for PSB 410A stock (20%; Figure 2.3A). Total leaf area was lowest prior to over winter cold storage for PSB 512A (393 cm^2) and BR P+1 (276 cm^2) stock (Figure 2.3B). For PSB 410A seedlings, total leaf area was lowest prior to field outplanting (344 cm^2). Maximum leaf area development after 30 d of growth occurred after 75 d of over winter cold storage for PSB 410A (402 cm^2) and PSB 512A (667 cm^2) seedlings and after 190 d of storage for BR P+1 (1249 cm^2) stock.

Stomatal conductance, after 30 d of growth, was affected by over winter cold storage duration ($P < 0.001$) and the interaction between over winter cold storage duration and stock type ($P < 0.001$). Stomatal conductance did not differ among stock types ($P = 0.664$). For container stock, stomatal conductance was highest prior to over winter cold storage (average 455 $\mu mol\ m^{-2}$

s^{-1}) and lowest after 150 d of over winter cold storage (average 218 µmol m^{-2} s^{-1}) (Figure 2.3C). For BR P+1 stock, stomatal conductance was highest after 190 d of over winter cold storage (353 µmol m^{-2} s^{-1}) and lowest after 75 d of cold storage (265 µmol m^{-2} s^{-1}).

The water potential of new stems produced after 30 d of growth in the growth chamber was influenced by stock type ($P < 0.001$), over winter cold storage duration ($P < 0.001$), and their interaction ($P = 0.014$). Prior to over winter cold storage, stem water potential was greater than -0.65 MPa for PSB 410A, PSB 512A, and BR P+1 stock (Figure 2.3D). For all nursery-grown stock, stem water potential values increased after 75 d of over winter cold storage (average -0.42 MPa). Stem water potential of container grown seedlings changed little between 75 and 150 d of over winter cold storage (average -0.39 MPa) whereas stem water potential of BR P+1 seedlings became more negative (-0.64 MPa) between 75 and 190 d of over winter cold storage.

Differences in root growth potential (RGP, expressed as root volume growth in ml) were due to over winter cold storage duration ($P < 0.001$) and its interaction with stock type ($P < 0.001$). RGP did not differ between stock types ($P = 0.883$). RGP was lowest prior to over winter cold storage for all stock types (average 7.2 ml) and highest after 75 d of over winter cold storage, especially for BR P+1 (33 ml) and PSB 512A (21 ml) stock (Figure 2.3E). Prior to field outplanting, the RGP of BR P+1 seedlings was 21 ml compared to 13 ml for PSB 512A stock and 10 ml for PSB 410A stock.

2.3.4. Field performance

After one growing season, all NR and BR P+1seedlings survived whereas survival was 90% for PSB 410A and 93% for PSB 512A stock. Leader and stem diameter growth, stem dieback, total leaf area, stem and root dry mass increment, and TNC varied by seedling type (all $P < 0.001$). Leader growth of NR seedlings was 75 cm compared to container and BR P+1 stock (average 39 cm) (Figure 2.4A). PSB 410A nursery stock had the most stem dieback (31%), while BR P+1 and NR seedlings had the least stem dieback (average 1%) (Figure 2.4B). Stem diameter growth was highest for NR seedlings (8.55 mm) and lowest for container and BR P+1 stock (average 3.52 mm) (Figure 2.4C). The NR seedlings produced the most total leaf area (1768 cm^2), while PSB 410A seedlings produced the least total leaf area (434 cm^2) (Figure 2.4D). Shoot dry mass growth was highest for BR P+1 seedlings (17 g) and lowest for PSB 410A stock (6 g) (Figure 2.4E). BR P+1 and NR seedlings produced the highest root dry mass growth (average 28 g) whereas container nursery stock produced seedlings with the least growth (average 13 g) (Figure 2.4E). One season after outplanting, stem TNC concentrations were highest for NR seedlings at 13.5 % of dry mass and lowest for PSB 512A stock at 11.1 % of dry mass (Figure

2.4F). Root TNC concentrations were highest for NR seedlings at 22.5% of dry mass and lowest for PSB 512A stock at 17.0 % of dry mass.

2.4. Discussion

2.4.1. Relationship between seedling condition at planting and first-year field performance

There were striking differences in morphology and physiology among the different seedling types prior to growth initiation during the spring of 2003. Nursery-grown container seedlings had an average root to shoot ratio (RSR) of approximately 1.0, bareroot P+1 (BR P+1) seedlings had a RSR of 2.0, and 'naturally' regenerating (NR) seedlings had a RSR of 6.6 (Table 2.1). In addition, NR seedlings had higher root starch reserves (15% of dry mass) compared with BR P+1 (10% of dry mass) and container (average 5% of dry mass) stock (Figure 2.1D). The relatively larger root system size and the higher levels of root carbohydrates are likely significant factors contributing to the much better growth of NR seedlings in 2003 (Figures 2.4A, C, and D) and possibly for the subsequent year; see the high TNC levels in NR seedlings at the end of the 2003 growing season (Figure 2.4F). BR P+1 stock followed the NR seedlings in RSR and root starch reserves levels and had better growth after outplanting compared to the container stock. In 2003, the NR seedlings grew the most; however, the allocation of carbon to root growth relative to shoot growth was highest in the BR P+1 stock (Figure 2.4E), likely in response to rebuilding the root system after pruning during the lifting process.

In north central Alberta, bud set of aspen growing under natural conditions generally occurs by mid-August and the first hard frost generally occurs by late September. In 2002, bud set in NR seedlings occurred by mid-July, a full month earlier than the BR P+1 stock growing under natural conditions at the bareroot nursery. The mechanism responsible for the early bud set in NR seedlings is not known; however, this early bud set likely resulted in a shift in carbon allocation from shoot growth to secondary growth of stems, to root growth, and to carbohydrate storage. The initiation of bud set in container stock while in the nursery occurred by mid-September, much later than both NR and BR P+1 seedlings. As a result, aspen container stock had less time between bud set and leaf senescence for secondary stem and root growth and carbohydrate reserve accumulation prior to lifting in December.

Interestingly, without root pruning at time of lifting, RSR and carbohydrate reserves in BR P+1 stock may have been even higher than those recorded. Pruning aspen roots shortened the length of coarse roots and removed many fine roots that are generally located distally from the stem (Ruark & Bockheim, 1987). Removal of fine roots probably decreased overall root

carbohydrate concentrations. In mature aspen, Landhäusser and Lieffers (2003) found that fine roots generally have higher sugar and starch concentrations during autumn than coarse roots.

High RSR and high carbohydrate reserves of aspen seedlings may be an important adaptation for establishment after outplanting. Young aspen stands of sucker origin have very high RSR (Peterson & Peterson, 1992), and therefore, are likely more tolerant of stresses (Perala & Russell, 1983). High root carbohydrate reserves are considered necessary for the growth of aspen root suckers after stem removal (Schier & Zasada, 1973; Landhäusser & Lieffers, 2002). Similarly, the RSR and carbohydrate reserve status characteristic of NR seedlings in this study may be an important adaptation against moisture stress. RSR has been used to differentiate between high and low quality planting stock of northern red oak (Tomlinson, Buchschacher, Teclaw, Colombo, & Noland, 1996) and a relationship between RSR and seedling survival after planting was found for *Eucalyptus camaldulensis* (Chamshama & Hall, 1987).

In addition to RSR and carbohydrate reserves, the increase in growth of NR seedlings relative to nursery-grown container and BR P+1 stock may be partly explained by the differences in planting dates. Germinants used to produce NR seedlings were planted in late June 2002 while nursery-grown container and BR P+1 stock were planted in mid-May 2003. These differences in planting dates affected the initiation of growth in 2003. The buds of NR seedlings flushed on 23 April whereas buds of nursery-grown container and BR P+1 stock did not flush until *ca* 10 d after planting. Bud set occurred at relatively the same time in mid-August in all seedling types. Therefore, the growing season of NR seedlings was approximately 35 d longer than nursery-grown stock. It is noteworthy that 13 d with night frosts occurred between 23 April and 28 May 2003. On nights with frosts, air temperatures did not decrease below –3.5°C and there was no visible damage to shoots.

2.4.2. Effects of over winter cold storage

Total non-structural carbohydrate (TNC) concentration of whole seedlings declined 9% of dry mass regardless of seedling type and is attributed to maintenance respiration. Roots had a steeper decline compared with stems (Figures 2.1E and F). This overall decrease in TNC exhibited by aspen seedlings due to over winter cold storage is consistent with findings for boreal coniferous tree species such as jack pine (*Pinus banksiana* Lamb.), black spruce (*Picea mariana* (Mill.) BSP) (Kim, Glerum, Hickie, & Chen, 1997), and white spruce (*P. glauca* Moench (Voss)) (Jiang, Zwiazek, & Macdonald, 1994; Wang & Zwiazek, 1999).

The pattern of root growth potential (RGP) was similar among stock types. After 10 d of cold storage, RGP increased and reached a maximum after 75 d (mid-January to late February) of

over winter cold storage (Figure 2.3E). This late winter peak in RGP in aspen appears to coincide with the period of ecodormancy (Lang et al., 1987), which is consistent with other deciduous tree species including European ash (*Fraxinus excelsior* L.), sessile oak (*Q. petraea* (Matt.)), and sycamore maple (*Acer pseudoplatanus* L.) (Mortazavi, O'Reilly, & Keane, 2004), and sugar maple (*A. saccharum* Marsh.), silver maple (*A. saccharinum* L.), and white ash (*Fraxinus americana* L.) (Farmer, 1975; Webb, 1977). There is a close association between RGP and shoot dormancy status. RGP is generally highest at the end of endodormancy when the buds have met their chilling requirements (Ritchie & Dunlap, 1980). Therefore, the chilling requirements of aspen seedlings used in this study were met after 1,800 hrs of storage at –3°C. Webb (1977) found that maximum RGP in silver maple, sugar maple, and white ash seedlings occurred after 3,000 to 3,500 hrs of chilling at +5°C.

After reaching this late winter maximum, RGP in aspen seedlings declined prior to field outplanting. A similar decline in RGP has been reported in European ash, sessile oak, and sycamore maple (Wood & Hanover, 1980; Mortazavi et al., 2004). In aspen, this decline in RGP may represent a shift in priority from root to shoot growth. In contrast to RGP, the time required to initiate bud flush in the RGP test was least prior to field outplanting after 150 d of storage for container stock and after 190 d of storage for BR P+1 stock (Figures 2.2A, B, and C). Further, the BR P+1 stock appears to have some of the best indicators of stem performance (i.e. highest leaf area and stomatal conductance, Figures 2.3B and C) at the end of the RGP test for seedlings that were cold stored for 190 d. This suggests that longer periods of storage are good for some aspects of shoot growth. However, the shift in priority from root to shoot growth may be problematic in aspen. Storage beyond about 75 d resulted in increased dieback of stems during the RGP test (Figure 2.3A), especially in the smallest stock type (PSB 410A). This dieback was also evident after outplanting (Figure 2.4B). Dieback of the stems after long storage times might be related to incomplete hardening of the stem prior to lifting or to decreased tolerance of cold temperatures due to dehardening of the stem after the chilling requirements of the buds had been met. Thus, for aspen seedlings, 75 d of cold storage at –3°C may represent a compromise between optimum RGP and decreased days to bud flush, declining TNC reserves, increased shoot growth, and increased stem damage with increasing time in cold storage.

In conclusion, the results of this study suggest that nursery-grown planting stock is morphologically and physiologically different from NR seedlings. In their first growing season, NR seedlings stopped height growth early, maintained their leaves, and then placed most of their carbon into root development and carbohydrate reserves, so that by late autumn these roots had large reserves of sugars and starches and substantial secondary growth. In contrast, seedlings

grown in nurseries had larger shoots, smaller roots systems, and lower carbohydrate reserves. In the first year after outplanting, nursery stock had less height and stem diameter growth and leaf area than the NR seedlings. It is not completely clear why nursery stock performed more poorly than the NR seedlings but it might relate to several factors. Container stock, in particular, might not have had a long enough period after bud set for secondary root growth and carbohydrate reserve accumulation in stems and roots prior to lifting. As a consequence, the quality of container seedlings was reduced by long-term over winter cold storage, which resulted in the lowest RGP and greatest levels of shoot dieback prior to outplanting. The BR P+1 seedlings appear to be intermediate between the NR seedlings and the container stock. They had the benefit of hardening out of doors, secondary stem and root growth, and carbohydrate reserve accumulation; however, root pruning at time of lifting may have set back the development of these seedlings after outplanting.

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Table 2.1. Mean (±SE) height, stem diameter, total (stem and root) dry mass, and root to shoot ratio (RSR) of PSB 410A (n = 40), PSB 512A (n = 40), bareroot P+1 (n = 10), and 'naturally' regenerating (n = 10) seedlings during the fall of 2002 prior to cold storage. 'Naturally' regenerating seedlings and container stock were one-year-old while bareroot P+1 stock was two-years-old.

Seedling type	Height (cm)	Stem diameter (mm)	Total dry mass (g)	RSR
'Natural'	3.2 ± 3.1 *c*[1]	1.21 ± 0.17 *d*	0.4 ± 0.4 *d*	6.6 ± 0.5 *a*
PSB 410A	31.7 ± 1.6 *b*	3.10 ± 0.08 *c*	1.1 ± 0.3 *c*	0.8 ± 0.1 *d*
PSB 512A	35.9 ± 1.6 *b*	3.96 ± 0.08 *b*	2.2 ± 0.3 *b*	1.2 ± 0.1 *c*
Bareroot P+1	51.0 ± 3.1 *a*	6.77 ± 0.17 *a*	15.5 ± 0.4 *a*	2.0 ± 0.5 *b*

[1] Means within columns followed by the same letter were not significantly different ($P \leq 0.05$) by DIFF option.

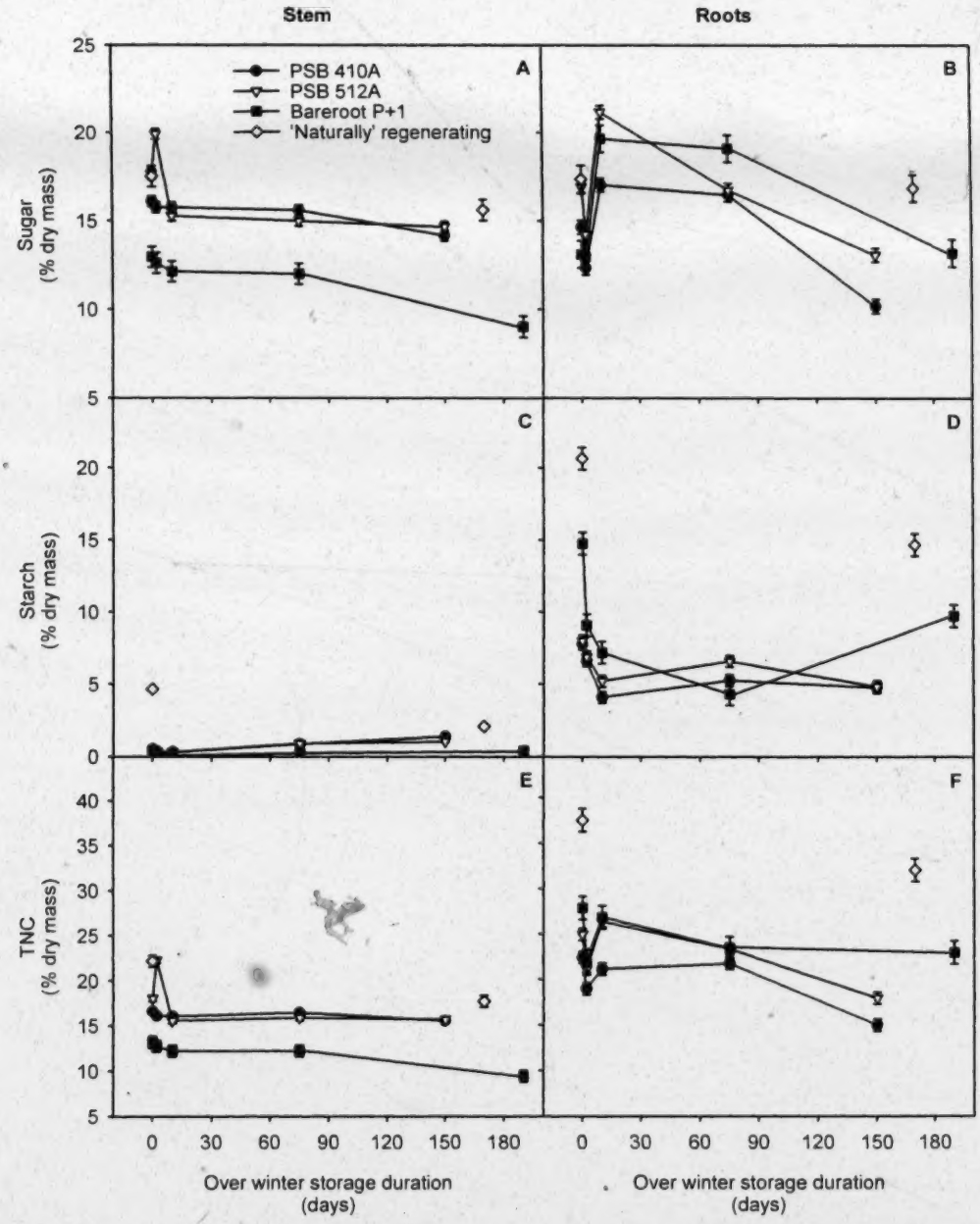

Figure 2.1. Mean (±SE) stem and root sugar (A and B), starch (C and D), and total non-structural carbohydrate (E and F) concentrations of aspen PSB 410A (n=40), PSB 512A (n=40), bareroot P+1 (n=10), and 'naturally' regenerating (n=10) seedlings in relation to duration of over winter cold storage.

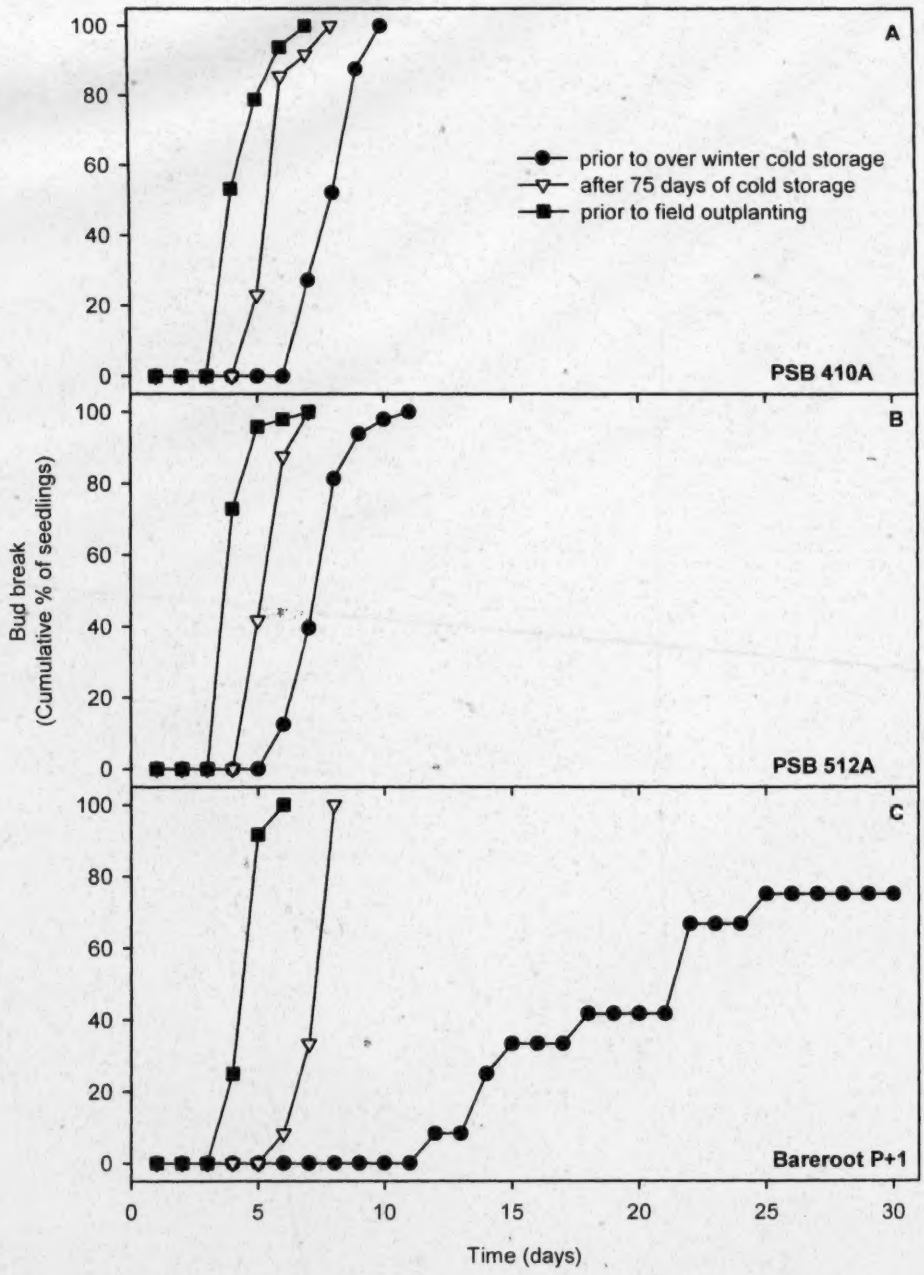

Figure 2.2. Days to initial bud flush of aspen PSB 410A (A, n=48), PSB 512A (B, n=48), and bareroot P+1 (C, n=12) seedlings prior to cold storage (-3°C), after 75 d of cold storage, and prior to field outplanting. Seedlings were checked daily and number with flushed buds recorded.

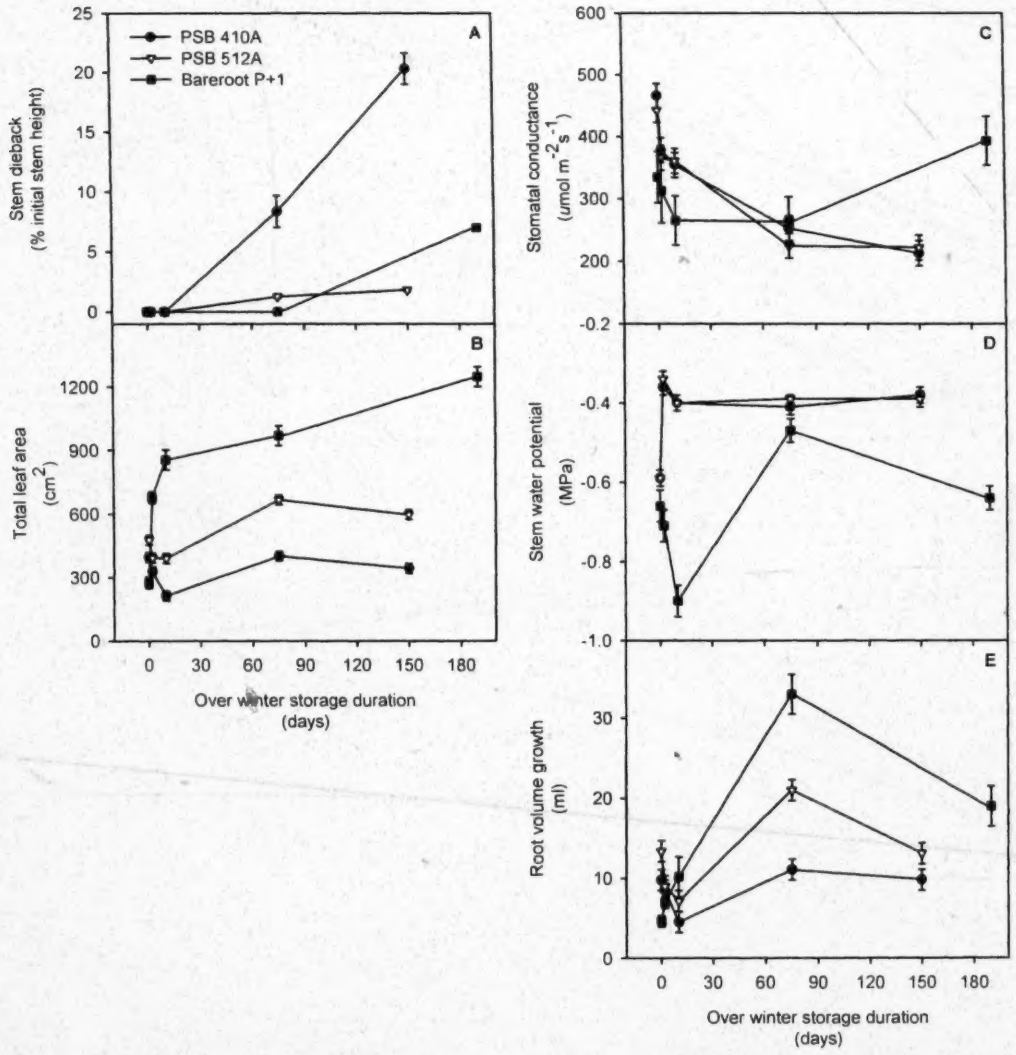

Figure 2.3. Mean (±SE) stem dieback (A), total leaf area (B), stomatal conductance (C), stem water potential (D), and root volume growth (E) of aspen PSB 410A (n=48), PSB 512A (n=48), and bareroot P+1 (n=12) seedlings after different intervals of cold storage at -3°C. All measurements were taken after 30 days growth in a growth chamber.

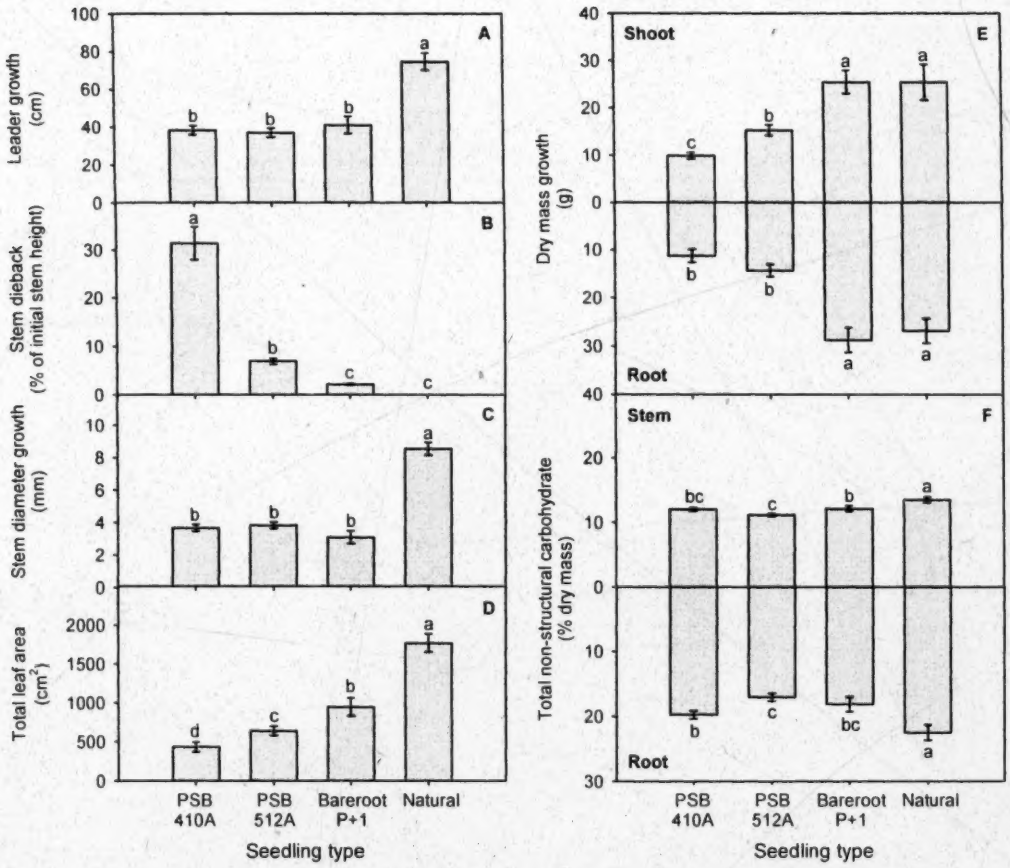

Figure 2.4. Mean (±SE) leader growth (A), stem dieback (B), stem diameter growth (C), total leaf area (D), shoot and root dry mass growth (E), and stem and root total non-structural carbohydrates (F) of aspen PSB 410A (n=40), PSB 512A (n=40), bareroot P+1 (n=10), and 'naturally' regenerating (n=10) seedlings one growing season after outplanting.

CHAPTER THREE

GROWTH OF ASPEN (*POPULUS TREMULOIDES* MICHX.) SEEDLINGS IN RESPONSE TO ROOT AERATION AND WATERING REGIME

3.1. Introduction

Aspen (*Populus tremuloides* Michx.) is an important source of wood fibre in the boreal forest (Peterson & Peterson, 1992). However, the long-term security of this wood supply is threatened by uncertainty around future harvest rights and land use conflicts with other resource based industries. As a result, there has been increased interest by industrial forest companies in plantation culture of aspen, aided by an availability of cleared agricultural land and the potential for tree improvement (Lester, 1995).

Currently, most aspen planting stock is grown as a summer crop in green houses under relatively controlled and stress-free environmental conditions (Burr, 1985; Johnson, 1997). Seedlings are commonly produced in large (220 to 336 ml) multi-cavity styroblock containers filled with peat based growth media amended with perlite and/or vermiculite. Seedlings are usually irrigated using an overhead boom system. However, the rapid growth of aspen seedlings, combined with high growing densities and a leaf canopy that limits water percolation to the roots, necessitates intensive irrigation (Burr, 1985).

Observations of containerized aspen seedling root systems grown in commercial nurseries find that most roots are located along container walls and there is limited root development in the centre of the plug. This suggests that there is higher oxygen and water availability to roots closer to container walls, and that periodic anaerobic conditions may exist in the centre of the plug. Oxygen availability to roots is affected by the physical characteristics of the growth medium (Bik, 1973; Bunt, 1983; Heiskanen, 1995) and is inversely related to soil water content (Bunt, 1961). Oxygen deficiency due to excess water in growth media reduces root growth in hardwood seedlings including aspen and balsam poplar (Landhäusser, Silins, Lieffers, & Liu, 2003), maple (Tripepi & Mitchell, 1984), birch (Tripepi & Mitchell, 1984; Landhäusser et al., 2003), and red ash (Sena Gomez & Kozlowski, 1980). Containerized aspen seedlings are also characterized by low root to shoot ratios (i.e. 1.0) and exhibit reduced survival and poor performance after outplanting (Chapter Two). This is in contrast to the high root to shoot ratios (i.e. 7.0), survival, and growth characteristic of aspen seedlings with unrestricted root systems (Chapter Two).

The objective of the experiment was to determine the importance of growth media aeration and watering regime as nursery cultural practices for improving root growth in container grown aspen seedlings. Improved root growth in containers may lead to increased root to shoot ratios, potentially enhancing aspen seedling establishment success after outplanting.

3.2. Materials and Methods

3.2.1. Growth media treatments

Growing media was a constant 1:1:1 (v:v) mixture composed of peat (Sunshine Peat Moss, Sun Gro Horticulture Canada Ltd., Seba Beach, Alberta), perlite (Terra-Lite 2000, Grace Horticultural Products, Ajax, Ontario), and vermiculite (medium grade, Grace Horticultural Products, Ajax, Ontario). Similar to the methods used by Bugbee and Frink (1986), five growth media with different aerations were produced by mixing coarse (C - unaltered peat, perlite, and vermiculite mix) and fine (F - produced by grinding the C mixture in a Willey mill to pass a 20-mesh sieve, particle size < 0.85 mm) fractions in different proportions. The proportions, expressed as percentage by volume, of F and C fractions were: 100F, 75F25C, 50F50C, 25F75C, and 100C. To improve growing conditions in the media, pH was adjusted from an average of 4.03 to 5.37 by adding 3.04 g dolomite (52.48% $CaCO_3$, 40.96% $MgCO_3$; Imasco Minerals Inc., Creston, British Columbia) $litre^{-1}$ of growth media (Desrochers, van den Driessche, & Thomas, 2003). The pH was determined from air-dried 10 ml samples (n = 5) mixed in 50 ml 0.01M $CaCl_2$ using an Accumet AP62 pH meter (Fisher Scientific Co., Ottawa, Ontario). Growth media fertility was not adjusted at time of mixing.

Polystyroblock trays (PSB 615A, cavity depth, 15.2 cm; diameter, 6.0 cm; and, volume, 336 ml; Beaver Plastics Ltd., Edmonton, Alberta) were cut into single containers and manually filled with the same volume of air-dried growth medium. To compact the medium, the filled container was dropped from a height of 10 cm from the ground surface three times. Medium was added to the top of the container and the procedure was repeated twice.

Bulk density, oxygen diffusion rate (ODR), air-filled porosity (AFP), and volumetric water content (WC) were determined using eight PSB 615A containers of each growth medium placed in a growth chamber. Over a six-week period, growth media were saturated with water from below three times per week. Containers were placed in 30 cm deep trays filled half full of water for 4 h, then removed and allowed to drain freely. To estimate bulk density, AFP, and WC, drainage holes were plugged and growth media were brought to saturation. Plugs were then removed and containers were covered with plastic to prevent water loss through evaporation and allowed to drain for 12 h to container capacity. Container weight was determined at growth

media saturation, at container capacity, and at constant mass after oven drying at 68°C for 72 h. Air-filled porosity was calculated by subtracting the weight of the container at capacity from the weight of the container at saturation. The difference was then divided by the weight of the container at saturation. Air-filled porosity was expressed as a percent. Growth media WC was determined by subtracting the weight of the container at capacity from the weight of the container at constant dry mass. The difference was then divided by the weight of the container at capacity. Growth media WC was expressed as a percent. The ODR was used to determine growth media aeration at container capacity. Using the platinum microelectrode method (Letey & Stolzy, 1964), ODR was measured at –7.5 cm (approximately the centre of the container) from the surface. Polarograms (McIntyre, 1970) were generated in a 1:1 mixture of peat and sand. Polarograms are amperage-voltage curves used to determine the appropriate voltage for ODR measurements. Voltage is applied in 0.1V increments from 0.1 to 0.9V. The current is read after 3 min with a 2 min rest period between voltage increments. The appropriate voltage for ODR measurements was determined at the point where the curve reaches a plateau. This plateau occurred at 0.45 V. ODR was calculated from the measured current (mA) and the surface area of the platinum electrode.

3.2.2. Plant material and culture

To improve germination success and uniformity of seedling size at the start of the experiment, seedlings were pre-established in small soft-walled containers. On 7 June 2004, aspen seeds, from a mixture of 10 open pollinated families of local origin (Edmonton, Alberta, 53° 34′ N 113° 25′ W, elevation 668 m), were sown into 200 peat based Jiffy 10 mm pellets (pellet depth, 2.0 cm; diameter, 1.0 cm; volume, 1.6 ml, Jiffy Products, Shippagan, New Brunswick). Pellets were placed in a growth chamber. After three weeks, twenty seedlings of relatively uniform height and stem diameter were transplanted into PSB 615A cavities containing each of the five growth media treatments, for a total of 100 seedlings. An additional 24 seedlings were transplanted into PSB 615A containers filled with 50F50C media. These seedlings were used to determine growth media water content and seedling weight over the course of the experiment (see *Water treatments* section below). Spacing between containers was 0.25 m × 0.25 m. Seedlings were watered immediately after transplanting and all growth media were maintained near container capacity. Growth chamber conditions throughout the experiment (total of 11 weeks) were 18/6 h day/night, 22/18°C day/night temperature and 60% relative humidity. Light was supplied by fluorescent lamps (Sylvania F48T12 SHA/VHO, Sylvania, USA) with 400 μmol m^{-2} s^{-1} photosynthetic active radiation at seedling height.

3.2.3. Water treatments

Fourteen days after transplanting, two watering regimes were superimposed on the growing media treatments, resulting in a total of 10 seedlings per growth medium and watering regime treatments. Watering treatments were determined gravimetrically (see Landis, Tinus, McDonald, & Barnett, 1989). The first watering regime (W1) had a target volumetric water content of 50% at the time of watering. To estimate the change in volumetric water content over time, four seedlings were randomly selected each week from the 24 additional seedlings grown in the 50F50C medium. The containers were weighed daily to determine water loss from the growth media. Weight of seedlings was determined on a weekly basis and used for weight adjustments in the following week. In the second watering treatment (W2), the time interval between watering determined for W1 was doubled. At each watering, growth media were brought to container capacity (approximately 70% volumetric water content in 50C50F treatment) by watering from below. Fertilization occurred when W1 and W2 were watered at the same time using a standard commercial nursery rate of 150 ppm N, 100 ppm P, and 160 ppm K (20-8-20 N-P-K with chelated micronutrients, Plant Products Co. Ltd., Brampton, Ontario; and, technical grade monopotassium phosphate 0-52-34 N-P-K, Haifa Chemicals Ltd., Haifa, Israel).

3.2.4. Water relations

At the final watering cycle, six weeks after superimposing the watering treatments, stomatal conductance was determined at 6 h intervals during daylight periods using a steady-state porometer (LI-1600, Li-Cor Inc., Lincoln, Nebraska). At the time of measurement, the final watering cycle was 18 h for W1 and 36 h for W2. Measurements were made at ambient light and temperature on the uppermost fully expanded leaf of ten seedlings for each watering and growth medium treatment combination. At the end of the watering cycle, four of the ten seedlings had water withheld for an additional 6 h and a final stomatal conductance measurement was taken at 24 h for W1 and 42 h for W2. After the final stomatal conductance measurements, the terminal 10 cm of the stem was excised to determine stem water potential using a Schölander pressure chamber (PMS Instruments, Corvallis, Oregon). Volumetric water content of the growth medium was also determined using a theta probe (Type ML2x, Delta Devices, Cambridge, England). Stem water potential and volumetric water content measurements were made on 6 seedlings at time 18 h (W1) and 36 h (W2) and 4 seedlings at time 24 h (W1) and 42 h (W2).

3.2.5. Growth measurements and carbohydrate status

Seedlings were measured for height and stem diameter 1 cm above the surface. After the physiological measurements, root volume (see *Carbohydrate status* section Chapter Two), projected leaf area (LI-3100, LI-COR, Inc., Lincoln, Nebraska), and root, stem, and leaf dry mass after oven drying at 68°C for 48 h were determined. Roots were separated into coarse (> 1.0 mm) and fine components. Shoot and root sugar and starch concentrations were determined using the methods described by Chow and Landhäusser (2004) (see *Carbohydrate status* section Chapter Two).

3.2.6. Experimental design and statistical analyses

The experiment was a 2 × 5 factorial split plot design with 2 watering regimes (W1 and W2) and 5 growth media aerations (100F, 75F25C, 50F50C, 25F75C, and 100C) as fixed effects and replicated 10 times. The watering regime formed the main plot while growth medium aeration formed the split plot. The treatment unit was a single seedling. Replicates were randomized on a weekly basis to compensate for the variation in growth chamber conditions.

Differences in stomatal conductance among treatments were tested by repeated measures analysis of variance (ANOVA) using SAS (SAS 8.1, SAS Institute, Cary, North Carolina) to account for correlation over time within individual seedlings. Because the time between measurements was not equal, the MIXED procedure was used. Compound symmetry provided the variance covariance matrix structure with the minimum AIC statistic (Wolfinger & Chang, 1995). Growth media physical properties and aspen seedling growth data were treated by ANOVA using the general linear models of SAS. To meet the assumptions of normality of the distribution of residuals and homogeneity of variance, stomatal conductance, height, leaf area, leaf dry mass, and root dry mass were log transformed while percentage data were arcsine square root transformed prior to analyses. Non-transformed means were used for presentation purposes. A significance level of $\alpha = 0.05$ was used for all analyses. When significant treatment effects were detected, Duncan's Multiple Range test was used to separate means.

3.3. Results

3.3.1. Growth media conditions

The growth medium with 100% coarse (C) fraction had an oxygen diffusion rate (ODR) of 13.0 $\mu g\ cm^{-2}\ min^{-1}$, which was significantly higher ($P < 0.001$) than the other four media with fine (F) fractions (Table 3.1). These four media had an average of 2.4 $\mu g\ cm^{-2}\ min^{-1}$. In addition, only the 100C fraction medium had significantly lower bulk density ($P < 0.001$) and water

content ($P < 0.001$) than the other four growth media (Table 3.1). The 100C also had significantly greater air-filled porosity (28%) compared to 9% for 100F ($P < 0.001$).

3.3.2. Water relations

Stomatal conductance over the 12, 18, and 24 h measurement periods was not affected by watering regime ($P = 0.573$), growth media ($P = 0.262$) or their interaction ($P = 0.922$) (Figure 3.1A). Stomatal conductance for the periods 36 and 42 h after watering for W2 was also not affected by growth media ($P = 0.256$). When comparing stomatal conductance at the end of the final watering cycle, however, stomatal conductance for W1 at 18 h was 99.0 mmol m^{-2} s^{-1} compared to 38.4 mmol m^{-2} s^{-1} at 36 hr for W2 (Figure 3.1A). Withholding water for an additional 6 h lowered the rate of decline in stomatal conductance in W2 by 25% compared to W1 ($P = 0.004$). Thirty percent of the seedlings grown under W2, regardless of growth media type, exhibited leaf wilting after withholding water for an additional 6 h. Stomatal conductance of these wilted seedlings averaged 16.3 mmol m^{-2} s^{-1}.

Stem water potential was affected by watering regime both at the end of the water cycle ($P < 0.001$) and after withholding water for an additional 6 h ($P < 0.001$). Stem water potential was higher for W1 at 18 h (-0.55 MPa) compared with W2 at 36 h (-1.17 MPa) (Figure 3.1B). After withholding water for an additional 6 h, stem water potential decreased by a further –0.03 MPa for W1 and –0.50 MPa for W2. There were no differences in stem water potential among growth media treatments at the end of the water cycle ($P = 0.795$) and after withholding water for an additional 6 h ($P = 0.453$).

There were differences in volumetric moisture content among watering regime and growth media treatments (watering regime × growth media interaction $P = 0.040$) at the end of the water cycle (data not shown). Volumetric water content was 29% higher for W1 compared with W2. Growth media with 50% or more F fraction had 6.5% higher volumetric moisture content compared with growth media with 75% or more C fraction. After withholding water for an additional 6 h, differences were only detected among watering regimes ($P < 0.001$) where volumetric water moisture content was 27% higher for W1 compared with W2.

3.3.3. Growth response

Overall, the seedlings in the W2 treatment produced 27% more fine root dry mass than seedlings in the W1 treatment ($P < 0.001$). With increasing proportion of C fraction in growth media, fine root dry mass decreased by 40% from 2.00 g to 1.21 g for W2 and by 34% from 1.42 g to 0.94 g for W1 (water regime ×growth media interaction $P = 0.025$) (Figure 3.2A).

Seedlings grown under the W2 watering regime had, on average, 5% larger stem diameter ($P = 0.029$), 17% greater leaf area ($P < 0.001$), and 5% lower specific leaf area ($P = 0.043$) compared with the W1 watering regime (Table 3.2). Shoot TNC concentrations were 2% higher in the W1 watering regime than in the W2 regime ($P < 0.001$). No differences between watering regimes were detected for seedling height, root volume, root to shoot ratio, and root TNC.

The media with the largest proportion of the F fraction (i.e. 100F and 75F25C) generally produced seedlings with the largest stem diameter, most leaf area, and highest root volume, and root to shoot ratio compared to seedlings in the media with larger proportions of the C fraction (Figures 3.2B, C, E, F). Specific leaf area was higher for seedlings grown in 100C and 25F75C media compared with those in 100F and 50F50C (Figure 3.2D). Growth media did not significantly affect seedling total height, and shoot and root TNC (data not shown).

3.4. Discussion

The results of this study suggest that seedling growth increased with decreasing air-filled porosity (AFP) and oxygen diffusion rate (ODR). This does not concur with other studies that found increased plant growth with increased substrate AFP (Biran & Eliassaf, 1980; Bugbee & Frink, 1986; Stowe, Lamhamedi, & Margolis, 2001; Wall & Heiskanen, 2003). Although the range of AFP in this study (9 to 28%) was in the range of substrate aerations used in other studies (1 to 40%), the differences in response to root aeration is likely due to the use of different plant species, growth media, and techniques to achieve the various aeration levels. In addition, the growth media tested in this study never reached anaerobic conditions. Although the AFP in the fine only medium was 9%, which is less than the 10% AFP often considered the lower limit for gaseous diffusion and root respiration (Xu, Nieber, & Gupta, 1992; Zou, Penfold, Sands, Misra, & Hudson, 2001), the oxygen diffusion rate in this medium was 1.6 $\mu g\ cm^{-2}\ min^{-1}$, which suggests the growth media treatments did not limit oxygen availability to roots. Typically, root growth is favoured when the ODR is > 0.7 $\mu g\ cm^{-2}\ min^{-1}$ and is severely limited at < 0.2 $\mu g\ cm^{-2}\ min^{-1}$ (Glinski & Stepniewski, 1985). Under severely waterlogged soil conditions (ODR <0.14 $\mu g\ cm^{-2}\ min^{-1}$), Landhäusser et al. (2003) observed an 80% reduction in aspen seedling water use and gas exchange compared with seedlings grown under well drained conditions reported in other studies (Landhäusser & Lieffers, 2001; Landhäusser, Desrochers, & Lieffers, 2001).

While the addition of fine particles to growth media has been shown to reduce air-filled porosity (AFP) to critical levels (Bunt, 1974; Handreck, 1983; Bugbee & Frink, 1986; Nkongolo & Caron, 1999), this study found no further reduction of AFP after the addition of 25% fine

fraction to the coarse material. This small change might be attributed to the filling of all macropores within growth media by the addition of fine particles (Spomer, 1979) resulting in relative continuity between pores (Allaire, Caron, Parent, Duchesne, & Rioux, 1996) regardless of fine particle proportion. Further, the fine particle size used in this study (< 0.85 mm) is at the lower limit of the range (0.8 mm to 6.0 mm) for peat moss particle size considered ideal for plant growth (Puutsjarvi & Richardson, 1975).

Fine root growth of aspen seedlings increased under a watering regime that allowed for temporary mild drought stress and in substrates with high fraction of fine particles. However, since the increased root growth occurred mainly in the fine root portion of the root system and was accompanied by an increase in leaf area and aboveground biomass, it did not result in higher root to shoot ratios. Increased amounts of fine particles in growth media are known to increase seedling growth (Bunt, 1983; Handreck, 1983; Bernier & Gonzalez, 1995). The large numbers of micropores in fine particles are able to hold more water than the macropores of coarse particles (Heiskanen, 1999; Nkongolo & Caron, 1999). Allocation of carbon to roots under mild moisture stress conditions and the resulting increase in leaf area growth and photosynthetic rates after the stress is alleviated has been well documented in the literature (Kramer, 1983; Tschaplinski, Tuskan, & Gunderson, 1994).

This study found that aspen seedlings grown in a growth chamber continued to transpire in response to high soil moisture deficits. After 6 h of additional drought treatment (Figure 3.1A), nearly one-third of aspen seedling grown under watering regime W2 reached the wilting point regardless of soil medium (data not shown). Of these, all seedlings had positive stomatal conductance. This result concurs with an earlier study on stomatal control of poplar in response to water deficits, which found that stomata of *P. trichocarpa* were unable to close despite desiccation and wilting (Hinckley & Braantne, 1994).

In conclusion, although increasing growth media aeration had little effect (within the range of this study) on root growth, additions of fine particle fractions to growth media increased fine root dry mass, stem diameter, leaf area, root volume, and root to shoot ratio. In addition, overall growth response of aspen seedlings was only marginally better to mild moisture stress (W2) than to over watering (W1). Therefore, this experiment suggests that root aeration and over watering do not appear to be factors causing poor root growth in aspen container stock grown in commercial nurseries. In these containers, aspen, with its high transpiration rates, used enough water, which resulted in adequate aeration (Bunt, 1983) even in growth media with high proportions of fine particles.

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Table 3.1. Comparison (mean ± SE, n = 8) of physical properties among growth media expressed as percent by volume of fine (F) and coarse (C) fraction. Measurements of oxygen diffusion rate, total porosity, and volumetric water content were taken at container capacity.

Growth Medium	Bulk density ($g\ cm^{-3}$)	Oxygen diffusion rate (ODR) ($\mu g\ cm^{-2}\ min^{-1}$)	Air-filled Porosity (AFP) (%)	Water Content (WC) (%)
100F	0.29±0.01 *a*[1]	1.6±0.1 *b*	8.7±0.7 *c*	58.2±1.3 *a*
75F25C	0.26±0.02 *a*	2.4±0.9 *b*	10.0±0.7 *bc*	59.2±1.7 *a*
50F50C	0.28±0.01 *a*	2.6±0.7 *b*	11.4±0.4 *b*	55.6±1.3 *a*
25F75C	0.26±0.02 *a*	3.8±1.1 *b*	11.8±0.6 *b*	57.6±1.9 *a*
100C	0.19±0.01 *b*	13.0±1.2 *a*	28.3±0.8 *a*	48.2±1.1 *b*

[1] Means followed by the same letter were not significantly different ($P \leq 0.05$) by Duncan's Multiple Range test.

Table 3.2. Effect of water regime (W1 and W2) on growth (mean ± SE, n = 50) of aspen seedlings after 11 weeks.

Growth response	Watering regime	
	W1	*W2*
Height (cm)	42.2±1.0 *a*[1]	42.9±0.9 *a*
Stem diameter (mm)	4.99±0.07 *b*	5.24±0.06 *a*
Total leaf area (cm^2)	683.7±20.5 *b*	825.9±16.5 *a*
Specific leaf area ($cm^2\ g^{-1}$)	162.7±3.3 *a*	154.9±2.0 *b*
Shoot dry mass (g)	5.55±0.13 *b*	7.06±0.17 *a*
Root dry mass (g)	1.94±0.06 *b*	2.54±0.08 *a*
Root volume (ml)	13.1±0.4 *a*	14.1±0.6 *a*
Root shoot ratio	0.35±0.01 *a*	0.36±0.01 *a*
Shoot TNC (% dry mass)	23.8±0.3 *a*	21.7±0.2 *b*
Root TNC (% dry mass)	12.0±0.5 *a*	11.3±0.4 *a*

[1] Means within rows followed by the same letter were not significantly different ($P \leq 0.05$) by Duncan's Multiple Range test.

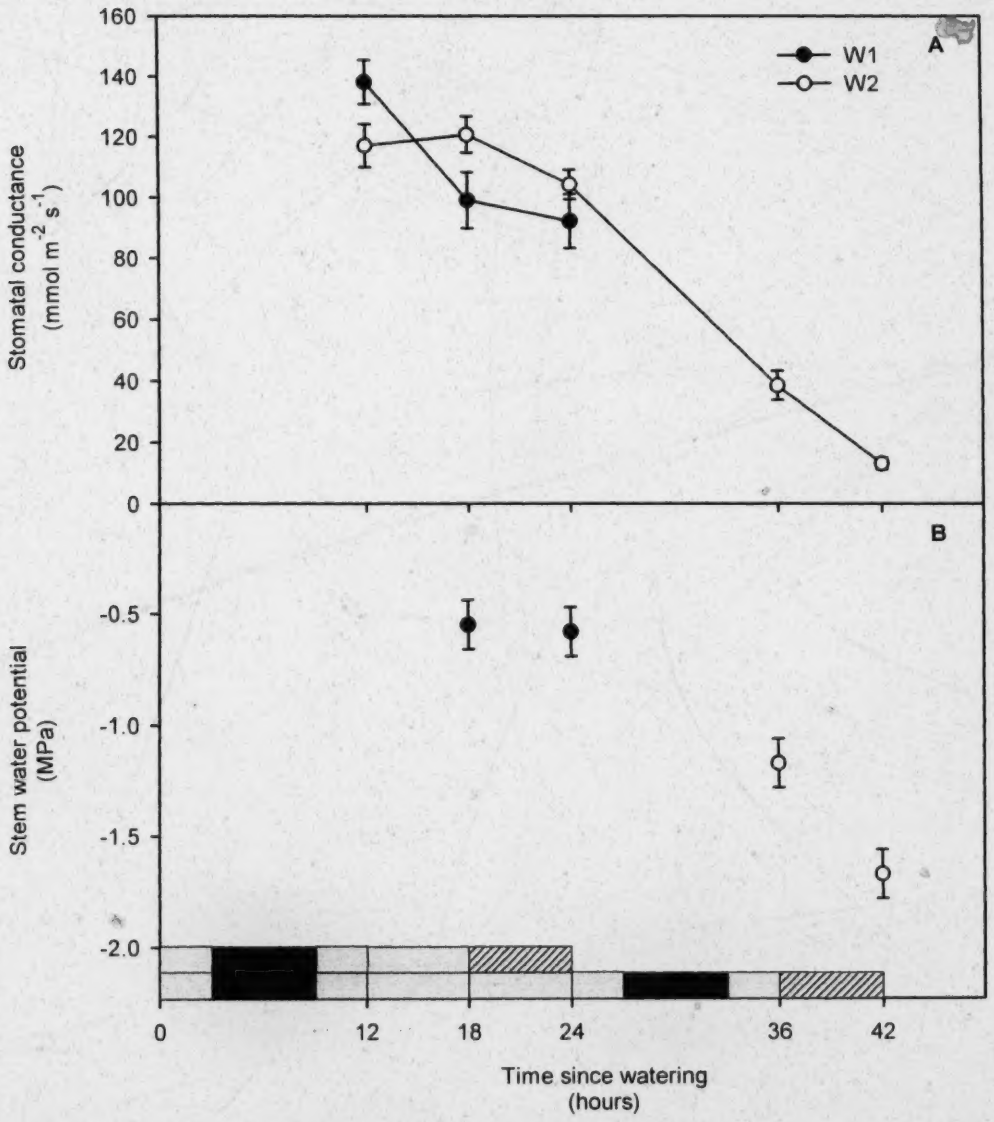

Figure 3.1. Mean (±SE) stomatal conductance (A) and stem water potential (B) of aspen seedlings subject to two watering regimes (W1 and W2). Measurements of stomatal conductance were taken every 6 h (12, 18, 24, 36, 42 h) of daylight (gray bars) from time of watering (0 h) during the last watering cycle for W1 (18 h) and W2 (36 h). Black bars represent 6 h of dark conditions. Water was withheld for 4 seedlings for an additional 6 h for both watering regimes (hatched bars).

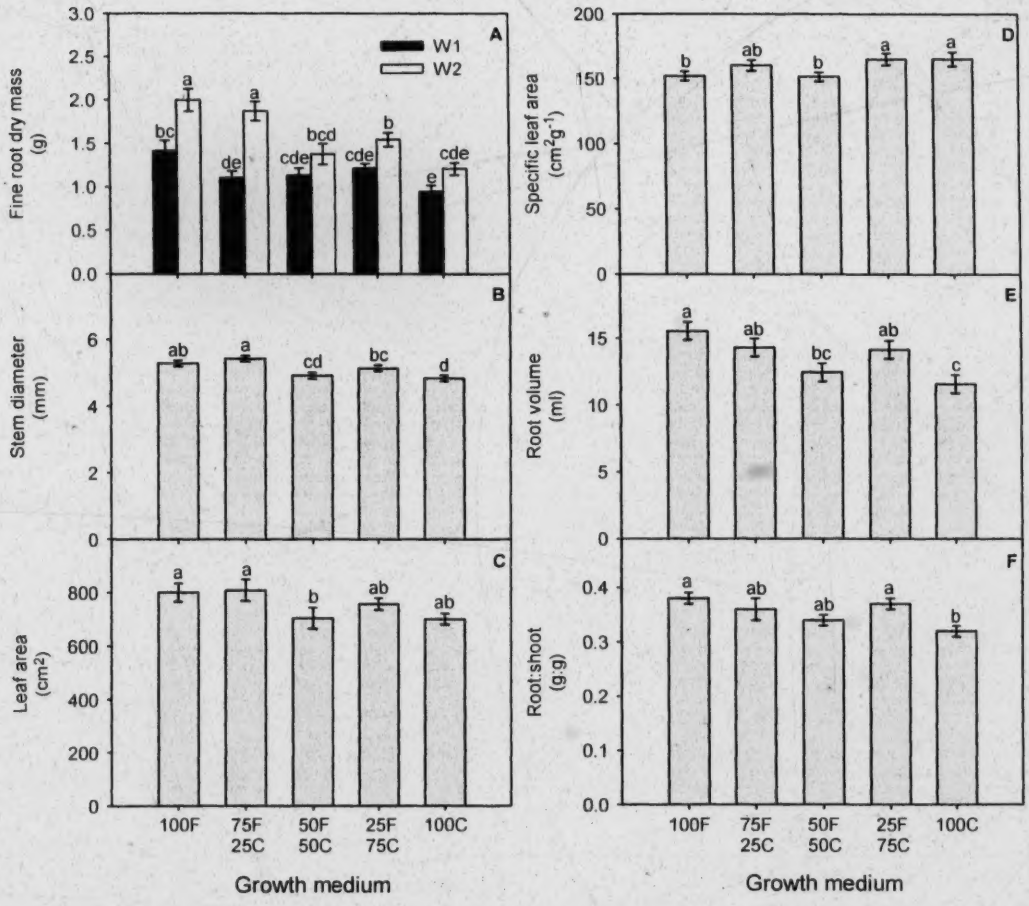

Figure 3.2. Average (±SE, n=10) fine root dry mass (A), stem diameter (B), leaf area (C), specific leaf area (D), root volume (E), and root to shoot ratio (F) of aspen seedlings after 11 weeks growth in media composed of different fine (F) and coarse (C) fractions, subject to two watering regimes.

CHAPTER FOUR

GENERAL CONCLUSIONS

4.1. Summary of results

The overall objective of the research was to advance the knowledge of nursery culture and over winter cold storage effects on aspen (*Populus tremuloides* Michx.) seedlings. This research is needed to improve overall survival and, in particular, growth of aspen planting stock in order to meet the objectives of forest management. Two experiments were performed. Experiment 1 examined the morphology and physiology, over winter cold storage tolerance, and first-year growth of nursery-grown and 'naturally' regenerating aspen seedlings (Chapter Two). Experiment 2 determined the growth of aspen seedlings in response to root aeration and watering regime (Chapter Three).

4.1.1. Differences in aspen seedling morphology and physiology

There were striking differences in morphology and physiology among seedling types. Prior to over winter storage, 'naturally' regenerating (NR) seedlings (i.e. one-year-old seedlings with unrestricted and unpruned root systems) had a root to shoot ratio (RSR) near 7.0 and root total non-structural carbohydrates (TNC) concentrations of 38% of dry mass. In contrast, nursery-grown bareroot P+1 (BR P+1) stock (i.e. two-year-old seedlings with pruned root systems) had a RSR of 2.0 and a root TNC concentration of 29% of dry mass while nursery-grown container (PSB 410A and PSB 512A) stock (i.e. one-year-old seedlings with root systems restricted by container walls) had an average RSR of 1.0 and a root TNC concentration of 24% of dry mass. These differences in RSR and root TNC concentrations among seedling types are likely related to the timing of bud set in the year prior to field outplanting. NR seedlings were grown out doors and initiated bud set by mid-July 2002 (Table 4.1). This resulted in the cessation of shoot growth early in the growing season (height 3.2 cm) followed by a long period (*ca* 70 d) between bud set and leaf senescence for root growth and carbohydrate accumulation. In contrast, nursery-grown container (PSB 410A and PSB 512A) and bareroot P+1 (BR P+1) stock had a longer period for shoot growth (height of BR P+1 stock was 51 cm while the height of container stock averaged 34 cm) and a shorter period (*ca* 40 d) between bud set and leaf senescence for root growth and carbohydrate accumulation (Table 4.1). The differences in RSR and root TNC concentration among nursery-grown stock types probably reflect differences in their growing environments. BR P+1 stock was grown out doors in a bareroot field and developed dormancy

and cold hardiness naturally while the container stock was grown under stress free environmental conditions and developed dormancy and cold hardiness artificially in a greenhouse. Therefore, dormancy and cold hardiness may be better developed in seedlings grown out doors compared with seedlings grown in a greenhouse. Interestingly, RSR and TNC concentrations of BR P+1 stock were likely even higher than the container stock prior to lifting; roots lost by BR P+1 stock during undercutting and pruning likely reduced their RSR and TNC concentrations.

4.1.2. Effects of over winter cold storage

The short-term response of nursery-grown aspen seedlings to freezing temperatures in over winter storage was to increase metabolic activity, to increase consumption of sugars, and to convert root starch to sugars. After 2 d of over winter cold storage, root sugar concentrations among stock types declined on average 3% of dry mass. After 10 d of over winter cold storage, root sugar concentrations among stock types increased 6% of dry mass from 13% to 19% of dry mass while root starch concentrations among stock types decreased 5% of dry mass from 10% to 5% of dry mass. Between lifting and planting in nursery-grown container and BR P+1 stock and between ground-freeze and ground-thaw in NR seedlings, whole seedling TNC concentrations decreased 9% of dry mass. This decline in seedling TNC concentrations was attributed to maintenance respiration, which was greater in roots (6% of dry mass) than in stems (3% of dry mass). Prior to outplanting in the spring of 2003, NR seedlings had the highest stem (18% of dry mass) and root (32% of dry mass) TNC concentrations while BR P+1 stock had the lowest stem TNC concentrations (9% of dry mass) and PSB 410A stock had the lowest root TNC concentration (15% of dry mass).

Nursery-grown aspen seedlings were dormant prior to over winter cold storage. Seedlings were leafless at time of lifting. The number of days to initiate bud flush was highest in BR P+1 stock (21 d) and container stock (9 d) prior to over winter storage and lowest prior to outplanting (*ca* 5 d in all stock types). Therefore, over winter storage is a useful practice in moving seedlings from endodormancy to ecodormancy in preparation for planting. This transition from endodormancy to ecodormancy, an indication of buds meeting their chilling requirements, occurred after 1800 hrs (75 d) of chilling at –3°C. However, stem dieback increased with increasing over winter storage duration in all nursery-grown stock types, especially in PSB 410A stock (20% of stem height). This suggests that seedlings were either not sufficiently cold hardy to withstand up to 190 d of cold storage or seedlings lost cold hardiness and, therefore, became less tolerant of cold temperatures while in cold storage.

Nursery-grown aspen seedling exhibited periodicity (Fuchigami & Nee, 1987) in root growth potential (RGP); however, this periodicity appears to be linked to the dormancy status of the buds. RGP was low prior to over winter cold storage when buds were endodormant. RGP peaked after 75 d of over winter cold storage and coincided with buds meeting their chilling requirements; however, holding seedling in ecodormancy resulted in a decline in RGP prior to outplanting in the spring. At the time of planting, RGP (measured as root volume growth) was highest in BR P+1 stock (21 ml) followed by PSB 512A (13 ml) and PSB 410A (10 ml) stock.

4.1.3. First-year growth of aspen seedlings after outplanting

The results clearly show that high RSR and high TNC concentrations prior to planting in the spring are necessary for high growth rates in aspen seedlings during the first growing season after outplanting. NR seedlings had a RSR of 7.0 and a total TNC concentration of 50% of dry mass prior to bud flush in the spring of 2003. At the end of the first growing season, these seedlings had the most height (75 cm) and stem diameter growth (8.6 mm) and most leaf area (1768 cm^2). In contrast, nursery-grown container and BR P+1 stock had RSR that ranged between 1.0 and 2.0 and total TNC concentrations of approximately 32% of dry mass. These seedlings grew 40 cm in height and 3.8 mm in stem diameter during the first growing season. NR seedlings and BR P+1 stock had similar shoot (25 g) and root (28 g) dry mass growth during 2003, which was double the amount of growth of container stock (shoot and root growth was 13 g dry mass). The high root growth of BR P+1 was attributed to the re-establishment of roots lost during undercutting and pruning at lifting. The high amount of growth of NR seedlings may be attributed to a longer growing season during 2003. Because NR seedlings were not planted in the spring, buds flushed on 23 April, which was 35 d earlier than the nursery-grown container stock (Table 4.1).

4.1.4. Growth of aspen in response to root aeration and watering regime

Both the oxygen diffusion rate and air-filled porosity decreased with increasing proportion of fine (F) fraction in growth media. Stomatal conductance was higher for seedlings watered regularly (W1) compared with seedlings that had the number of waterings reduced by half (W2) compared with W1 at the end of the final water cycle (18 h W1 and 36 h W2) and after withholding water for an additional 6 h. After 11 weeks, seedlings grown in media with a large F fraction had greater fine root dry mass, stem diameter, root volume, and RSR compared with those grown in media with a large C fraction. Seedlings grown under W2 has greater stem diameter, leaf area, and shoot and root dry mass compared with seedlings grown under W1. In

growth chamber conditions, growth media with >75% F fraction by volume produced larger containerized aspen seedling especially under reduced watering.

4.2. Implications for forest management

Silviculturalists have traditionally relied on seedling stem height and diameter specifications as the criteria for making payment to commercial nurseries for growing seedlings and as a means to assess seedling quality. Seedlings are culled from orders if they do not meet the predetermined height and stem diameter specifications. It is clear that seedling morphology (RSR) is an important aspect of quality in aspen (Chapter Two); however, the results also suggest that seedling physiology (carbohydrate reserves) is equally important. Therefore, silviculturalists must recognize a need to develop seedling quality specifications based on both morphological and physiological attributes. Silviculturalists must also recognize that the evaluation of seedling quality is intimately linked to nursery practices and to field performance.

Aspen seedlings used for plantation establishment have traditionally been summer grown, fall-lifted, and over winter cold stored prior to spring planting. Results suggest that the chilling requirements of aspen seedlings are met after 75 d of over winter cold storage (Chapter Two). Over winter storage beyond 75 d resulted in a deterioration of seedling quality (e.g. an increase in stem dieback and a decrease in carbohydrate reserves and RGP). If spring planting programs are to be maintained, then nursery managers and silviculturalists may want to consider the winter production of aspen planting stock. Under this production regime, seeds are sown in early December and seedlings are grown until mid-January, hardened off in preparation for lifting, and over winter cold stored beginning in late February. This regime is only applicable for container stock. The benefit of this production regime is that seedlings will be at their maximum root growth potential at time of planting, which would likely increase plantation establishment success. In addition, the cooler temperatures in the greenhouse during winter may be beneficial for increasing RSR. Odlum and Ng (1995) found that root growth was favoured over shoot growth when greenhouse day/night temperatures were 21°C/19°C for black spruce and 19°C/14°C for jack pine. However, under a winter production regime, seedling costs may increase due to increased need for greenhouse heating and artificial lighting. If summer production schedules for aspen seedlings are to be maintained, then silviculturalists may want to consider an autumn planting program. Seedlings that are dormant and properly hardened (i.e. most tolerant of stresses) could be planted soon after lifting in the autumn rather than in the spring when seedlings are no longer dormant and susceptible to handling and planting stresses. Under an autumn planting regime, current nursery production of BR P+1 stock could be

maintained; however, container stock would have to be sown earlier (May) than current practices and placed outside in mid-July to develop dormancy and cold hardiness under more natural conditions prior to planting in mid-October.

An irrigation strategy was developed for containerized aspen seedlings grown in media with a 50F50C particle size fraction during the establishment and rapid growth phases (Landis, Tinus, McDonald, & Barnett, 1992) (Figure 4.1). To promote seed germination and early growth of aspen in containers, substrate water content should be maintained near container capacity during the establishment phase (Figure 4.1). However, during the rapid growth phase aspen seedlings can be produced with substrate moisture content ranging from 9% (v:v) to 70% (v:v) (average water content 42% v:v) without compromising seedling growth, morphology, and carbohydrate accumulation (Chapter Three). This irrigation strategy has implications for water management. There is ever increasing demand for water. Efficient use of water in nurseries can reduce the costs associated with seedling production and also increase the longevity of irrigation and fertilization equipment. In addition, if less water is applied to seedlings, then there will be fewer minerals leached from the substrate into ground water sources or discharged into watercourses.

4.3. Limitations of the experiments

RGP tests and field performance comparisons among aspen seedling types were conducted under optimal conditions where nutrients and/or water were likely not limiting growth (Chapter Two). Although growing seedlings under these conditions allows for direct comparisons of maximum growth potential among seedlings types, they do not necessarily reflect the growth potential of seedlings under field conditions where seedlings may experience low soil temperature and/or moisture (Ritchie, 1985; Grossnickle, Arnott, Major, & Tschaplinski, 1991). Testing seedlings under these conditions may be a more realistic measure of their quality or 'fitness for purpose'.

Growth of aspen seedlings was evaluated one growing season after outplanting (Chapter Two). To properly assess seedling performance after outplanting, experiments should extend longer than one growing season. Previous studies on growth of aspen seedlings in plantations report results after at least three growing seasons (e.g. Benson & Einsphar, 1964; Reighard, Howe, & Hanover, 1985; van den Driessche, Rude, & Martens, 2003). In order to make definitive statements about the performance among the seedling types used in this study, seedling growth should have been monitored for at least three growing seasons (i.e. during the seedling establishment phase).

Containerized seedlings generally undergo six developmental stages prior to outplanting: germination, early growth, rapid growth, bud initiation, stem finishing, and cold conditioning (Landis, Tinus, McDonald, & Barnett, 1992). The effects of root aeration and watering regime were studied in aspen seedlings during the germination, early growth, and rapid growth phases (Chapter Three). The experiment did not determine the response of aspen seedlings to root aeration and watering regime during the bud initiation, stem finishing, and cold conditioning phases. Along with a reduction in photoperiod and fertilization rates, especially nitrogen, water is greatly reduced or withheld for a period of time to initiate bud set in aspen. Because growth is reduced, seedling requirements for water may also decrease after bud set and during cold conditioning. Therefore, the irrigation strategy developed for seedlings during the germination, early growth, and rapid growth phases (Figure 4.1) may not be appropriate for seedlings during the bud initiation, stem finishing, and cold conditioning phases. It is also not clear how seedlings grown in substrates of different aeration would be affected by over winter cold storage or how they would perform after outplanting. Studies have shown that seedlings with substrates of higher aeration may have air gaps caused by poor root-soil contact, which can lead to greater resistance to water flow from soil to roots after planting (Kramer, 1983; Grossnickle, 2005).

4.4. Future directions

The experiments conducted as part of this thesis provide some baseline information on aspen seedling quality as it relates to outplanting performance. Much information about aspen seedling production, quality, and outplanting performance is still needed. Root to shoot ratio (RSR) and carbohydrate status are important factors that help aspen seedlings grow well; however, there is a need to better understand the relationship between RSR and carbohydrate status and performance under various field conditions. In addition, if silviculturalists insist on having aspen seedlings with high RSR and carbohydrate reserves as part of their planting programs, then it is up to nursery growers to explore ways to meet these specifications. Seedlings with high RSR and high carbohydrate reserves were barerooted, grown out doors, and had set bud early in the growing season (Chapter Two). The mechanism responsible for the early bud set in these seedlings and the relationships between timing of bud set and RSR and carbohydrate status are not known. It is also not clear whether high RSR and high carbohydrate reserves are possible for aspen seedlings grown in containers.

Root growth potential (RGP) and carbohydrate reserves were used to evaluate the quality of aspen seedlings (Chapter Two); however, other tests such as electrolyte leakage (O'Reilly, Harper, & Keane, 2002), chlorophyll fluorescence (Mohammed, Binder, & Gillies, 1995),

nutrient status (Jozefek, 1989), and stress-induced volatile emissions (Templeton & Colombo, 1995) may prove beneficial in assessing aspen seedling quality and are worthy of consideration. These physiological tests quantify the stress resistance, dormancy status, and cold hardiness of seedlings. Nursery managers can use these techniques to better determine when to lift and how long to over winter cold store aspen seedlings prior to field planting.

4.5. Literature cited

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Table 4.1. Timeline of aspen seedling growth at different phenological stages during nursery operations and in the field (from Chapter Two).

	PSB 410A	PSB 512A	Bareroot P+1	'Naturally' regenerating
Nursery (2002)				
Shoot elongation (duration)[1]	July - September (75 d)	July - September (75 d)	May - August (80 d)	June - July (35 d)
Bud set	late- September	late-September	mid-August	mid- July
Leaf senescence (duration)[2]	mid-November (40 d)	mid-November (40 d)	late-September (40 d)	late-September (70 d)
Lifting date	10 December	10 December	19 October	Not lifted
Over winter storage length	150 d	150 d	190 d	180 d
Chilling requirement	75 d (1800 hrs)	75 d (1800 hrs)	75 d (1800 hrs)	?
Field (2003)				
Planting date	18 May	18 May	18 May	Not planted
Bud flush	28 May	28 May	28 May	23 April
Shoot elongation (duration)[1]	May to August (80 d)	May to August (80 d)	May to August (80 d)	April to August (115 d)
Bud set	mid-August	mid-August	mid-August	mid-August

[1]Length of time between germination or bud flush and bud set.

[2]Length of time between bud set and leaf senescence for root growth and carbohydrate accumulation.

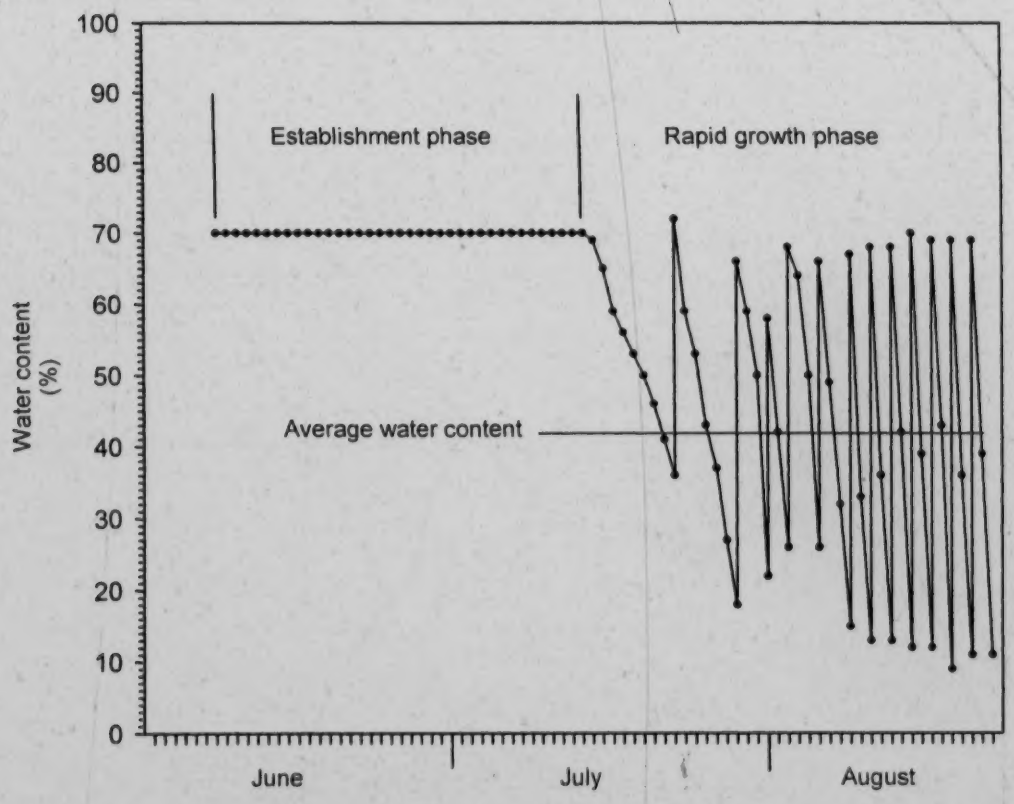

Figure 4.1. Proposed irrigation strategy for containerized (PSB 615A) aspen seedlings grown in 50F50C media during the germination, establishment, and rapid growth phases under growth chamber conditions (Chapter Three).